THE UNIVERSITY OF LIVERPOOL

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by

PROFESSOR WARRINGTON YORKE, M.D., F.R.C.P., F.R.S.

PROFESSOR D. B. BLACKLOCK, M.D., D.P.H.

PROFESSOR R. M. GORDON, O.B.E., M.D., Sc.D., M.R.C.P., D.P.H.

DR. T. SOUTHWELL, D.Sc., Ph.D., F.R.S.E.

PROFESSOR T. H. DAVEY, M.D., D.T.M.

VOLUME 32

(April 28th, 1938, to December 21st, 1938)

With Frontispiece, ten plates, and ninety-six figures in text

LIVERPOOL:
THE UNIVERSITY PRESS OF LIVERPOOL

H. R. GRUBB, LTD.,
PRINTERS,
POPLAR WALK, CROYDON.

Medical
Fabian

CONTENTS

No. 1. April 28th, 1938

LEAGUE OF NATIONS HEALTH ORGANISATION — PAGE

Treatment of malaria: study of the therapeutics and prophylaxis of malaria by synthetic drugs as compared with quinine: fourth general report of the Malaria Commission 1

GIBBINS, E. G.
The mouth-parts of the female in *Simulium damnosum* Theobald, with special reference to the transmission of *Onchocerca volvulus* Leuckart 9

GIBBINS, E. G.
Notes on Ethiopian Simuliidae, II 21

PAWAN, J. L.
An unusual strain of rabies virus in a vampire bat 35

BLACKLOCK, D. B.
Protection for the roofs of buildings in the tropics 39

CHRISTOPHERS, SIR S. RICKARD; and FULTON, J. D.
Observations on the respiratory metabolism of malaria parasites and trypanosomes 43

FULTON, J. D.; and CHRISTOPHERS, SIR S. RICKARD
The inhibitive effect of drugs upon oxygen uptake by trypanosomes (*Trypanosoma rhodesiense*) and malaria parasites (*Plasmodium knowlesi*) 77

SOUTHWELL, T.; and KIRSHNER, A.
On the transmission of leishmaniasis 95

GLYN-HUGHES, F.; LOURIE, E. M.; and YORKE, WARRINGTON
Studies in chemotherapy. XVII: The action of undecane diamidine in malaria 103

CONTENTS

No. 2. August 2nd, 1938

PAGE

BLACKLOCK, D. B.
A device for applying oil or other liquids to flushing cisterns 109

HO, CH'I
On some species of *Sarcophaga* from Java and its neighbouring islands ... 115

KAU, L. S.; and WU, KUANG
A note on the pathology of schistosomiasis due to *S. japonicum* among cattle in China 129

KAU, L. S.; and WU, KUANG
Pathological findings among pigs experimentally infected with *Fasciolopsis buskii* 133

DAENGSVANG, SVASTI; and TANSURAT, PRADIT
A contribution to the knowledge of the second intermediate hosts of *Gnathostoma spinigerum* Owen, 1836 137

PATTON, W. S.; and HO, CH'I
The study of the male and female terminalia of the genus *Sarcophaga*, with illustrations of the terminalia of the *haemorrhoidalis* group... 141

KIRSHNER, A.
A new species of nematode (*Subulura leachii*) from a kingfisher... 159

DEVINE, J.
Studies in chemotherapy. XVIII: Changes in the blood and urine produced by administration of undecane diamidine 163

KING, H.; LOURIE, E. M.; and YORKE, WARRINGTON
Studies in chemotherapy. XIX: Further report on new trypanocidal substances 177

SOUTHWELL, T.; and KIRSHNER, A.
Some observations on guinea-worm larvae 193

CORSON, J. F.
The cerebrospinal fluid of monkeys (*Cercopithecus* sp.) infected with a strain of *Trypanosoma rhodesiense* 197

LOURIE, E. M.; and YORKE, WARRINGTON
Studies in chemotherapy. XX: The preparation of strains of trypanosomes resistant to synthalin and undecane diamidine, and an analysis of their characters 201

CONTENTS

No. 3. October 12th, 1938

	PAGE
WEINMAN, DAVID ; and PINKERTON, HENRY A bartonella of the guinea-pig, *Bartonella tyzzeri* sp.nov.	215
CABALLERO Y C., EDUARDO Nematodes of the reptiles of Mexico, II	225
WALKER, A. J. Fungal infections of mosquitoes, especially of *Anopheles costalis*	231
CORSON, J. F. A third note on a strain of *Trypanosoma gambiense* transmitted by *Glossina morsitans*	245
FOY, HENRY ; and KONDI, ATHENA A note on intracorpuscular methaemoglobin in plasmochin toxicity	249
CHRISTOPHERS, SIR S. RICKARD ; and FULTON, J. D. Observations on the course of *Plasmodium knowlesi* infection in monkeys (*Macacus rhesus*), with notes on its treatment by (1) atebrin and (2) 1 : 11 normal undecane diamidine, together with a note on the action of the latter on bird malaria	257
HO, CH'I A new tree-hole breeding *Anopheles* from the island of Hainan...	279
HO, CH'I The significance of the female terminalia of house-flies as a grouping character	287
HAWKING, FRANK Analysis of the trypanocidal action of trivalent arsenicals and acriflavine ...	313

CONTENTS

No. 4. December 21st, 1938

	PAGE
CORKILL, N. L. Arcus senilis as a sign of chronic vitamin C deficiency	333
KIRK, R. A laboratory study of Abyssinian louse-borne relapsing fever	339
KIRK, R. The non-transmission of Abyssinian louse-borne relapsing fever by the tick *Ornithodorus savignyi* and certain other blood-sucking arthropods... ...	357
HAWKING, FRANK A quantitative study of the photosensitivity induced in trypanosomes by acriflavine	367
HAWKING, FRANK A note on the trypanocidal action of atebrin in relation to its absorption by trypanosomes	383
HO, CH'I On a collection of anopheline mosquitoes from the island of Hainan	387
DAVEY, T. H.; and GORDON, R. M. The size of aperture necessary in screencloth intended for the protection of dwellings in West Africa	413
BINNS, H. R. Observations on the behaviour in laboratory animals of *Trypanosoma congolense* Broden, 1904	425
BERTRAM, D. S. A note upon myiasis due to the larvae of *Cordylobia rodhaini* Gedoelst ...	431
CORSON, J. F. A record of some complications which occurred in the course of experimental infections of African volunteers with *Trypanosoma rhodesiense*	437
MARY KINGSLEY MEDALLISTS	i
PRESENTATION OF MARY KINGSLEY MEDALS	ii
INDEX	xii

12 3.1938

April, 1938 Vol. 32. No. 1

Annals of Tropical Medicine and Parasitology

ISSUED BY
THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Natural formula

Essentials to effective leprosy control

IODISED 'MOOGROL'

TRADE MARK

A Mixture of Esters of Acids of the Chaulmoogric Series with 0·5 per cent. Iodine

Relatively free from general and local reactions. Suitable for intradermal and intramuscular injection.

Bottles of 25 c.c. at 3/- each, and 100 c.c. at 10/- each
Bottles of 5 litres at 75/- each

TRADE MARK 'MOOGROL'

Mixture of Esters of Acids of the Chaulmoogric Series

Bottles of 25 c.c. at 3/- each, and 100 c.c. at 10/- each
Bottles of 1 litre at 45/- each

London Prices in Pre-War Packings

BURROUGHS WELLCOME & CO.
LONDON

D.777

Volume 32 April 28th, 1938 No. 1

ANNALS

OF

TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY

THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

EDITED BY

PROFESSOR WARRINGTON YORKE, M.D., F.R.C.P., F.R.S.

PROFESSOR D. B. BLACKLOCK, M.D., D.P.H.

PROFESSOR R. M. GORDON, M.D., Sc.D., M.R.C.P., D.P.H.

DR. T. SOUTHWELL, D Sc., Ph.D.. F.R.S.E.

THE INCORPORATED
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

FOUNDED BY SIR ALFRED LEWIS JONES, K.C.M.G.

(Affiliated with the University of Liverpool)

Patron : HIS MAJESTY THE KING

Hon. President :

Hon. Vice-Presidents : THE EARL OF DERBY, K.G., G.C.V.O., C.B., LL.D.
SIR EDWARD MEREWETHER, K.C.V.O.
SIR H. J. READ, K.C.M.G.

Chairman : THE VISCOUNT LEVERHULME

Vice-Chairman : Mr. H. D. DICKIE

COUNCIL

THE VICE-CHANCELLOR OF THE UNIVERSITY OF LIVERPOOL — *University of Liverpool*

THE VISCOUNT LEVERHULME
Professor H. E. ROAF, M.D., D.Sc., L.R.C.P., M.R.C.S. — *Council of University of Liverpool*

Professor W. H. WOOD, B.Sc., M.D.
Professor H. D. WRIGHT, B.A., M.D., D.Sc., M.R.C.P.E. — *Senate of University of Liverpool*

Mr. F. G. HEISE
Mr. PICTON H. JONES — *Liverpool Steamship Owners' Association*

Mr. J. N. SELLERS, *Shipowners' Association*

Mr. T. EDWARD LESCHER, O.B.E.
Mr. ARTHUR D. DEAN — *Liverpool Chamber of Commerce*

Professor D. B. BLACKLOCK, M.D.
Mr. G. BROCKLEHURST
Mr. J. R. DANSON, M.C.
Professor R. M. GORDON, M.D.
Sir RICHARD D. HOLT, Bart., J.P.
Mr. ROBERT L. HOLT
Mr. J. PICKERING JONES
Mrs. PERCY F. KIPLING
Sir JOHN NICHOLSON, Bart.
Sir ROBERT RANKIN, Bart., M.P.
Dr. T. SOUTHWELL, D.Sc., Ph.D., F.R.S.E.
Professor W. YORKE, M.D., F.R.C.P., F.R.S.

Mr. J. R. HOBHOUSE, M.C., *Hon. Treasurer*

Mr. J. L. MCCARTHY, F.C.I.S., *Hon. Secretary*, The Chamber of Commerce, 1, Old Hall Street, Liverpool, 3.

STAFF

TROPICAL MEDICINE :

Alfred Jones Professor of Tropical Medicine :
WARRINGTON YORKE, M.D., F.R.C.P., F.R.S.

Lecturer in Tropical Medicine :
A. R. D. ADAMS, M.D., D.T.M.

Tropical Surgery :
PROFESSOR R. E. KELLY, C.B., F.R.C.S.

Tropical Dermatology :
R. STOPFORD-TAYLOR, D.S.O., M.B., Ch.B., F.R.C.S.E.

Tropical Ophthalmology :
A. McKIE REID, M.C., M.B., Ch.B., F.R.C.S.

TROPICAL HYGIENE :

Professor of Tropical Hygiene :
D. B. BLACKLOCK, M.D., D.P.H.

Practical Sanitation :
PROFESSOR W. M. FRAZER, M.D., D.P.H.

Public Health Chemistry :
ASSOCIATE PROFESSOR W. H. ROBERTS, M.Sc., F.I.C.

Sanitary Engineering :
PROFESSOR R. G. BATSON, A.K.C., M.Inst.C.E., M.I.Mech.E.

Lecturer in Vital Statistics :
C. O. STALLYBRASS, M.D., D.P.H.

ENTOMOLOGY :

Dutton Memorial Professor of Entomology :
R. M. GORDON, M.D., Sc.D., M.R.C.P., D.P.H.

Assistant Lecturer and Demonstrator in Entomology :
D. S. BERTRAM, B.Sc.

PARASITOLOGY :

Lecturer in Charge of the Department :
T. SOUTHWELL, D.Sc., Ph.D., F.R.S.E.

Assistant Lecturer in Parasitology :
A. KIRSHNER, M.B., Ch.B., D.T.M.

Honorary Assistant Lecturer in Veterinary Parasitology :
G. O. DAVIES, M.V.Sc., M.R.C.V.S., D.V.H.

BACTERIOLOGY :

Professor of Bacteriology :
H. D. WRIGHT, B.A., M.D., D.Sc., M.R.C.P.E.

RESEARCH FELLOWSHIPS :

Caton Memorial Fellow :
Vacant.

Chemotherapy : Medical Research Council Research Workers :
E. M. LOURIE, M.B., B.S., D.P.H., D.T.M. & H.
J. DEVINE, B.Sc., Ph.D.

Junior Tropical Medical Research Fellow, Medical Research Council :
J. L. DALES, M.B., Ch.B.

MUSEUM :

Curator :
MARY G. BLACKLOCK, B.Sc., M.B., Ch.B., B.A.O., D.T.M.

LIBRARY :

Librarian :
GLADYS PHILLIPS, M.A.

ROYAL INFIRMARY : TROPICAL DEPARTMENT :

Physician :
PROFESSOR WARRINGTON YORKE, M.D., F.R.C.P., F.R.S.

Assistant Physician :
A. R. D. ADAMS, M.D., D.T.M.

Consulting Surgeon :
PROFESSOR R. E. KELLY, C.B., F.R.C.S.

Consulting Dermatologist :
R. STOPFORD-TAYLOR, D.S.O., M.B., Ch.B., F.R.C.S.E.

Consulting Ophthalmologist :
A. McKIE REID, M.C., M.B., Ch.B., F.R.C.S.

SIERRA LEONE RESEARCH LABORATORY :

Professor of Tropical Diseases of Africa :

Assistant Director :
T. H. DAVEY, M.D., D.T.M.

Leverhulme Research Assistant :
A. J. WALKER, M.D., C.M., D.T.M.

Junior Tropical Medical Research Fellow, Medical Research Council :
G. B. LUDLAM, M.B., Ch.B., D.T.M., D.T.H.

THE INCORPORATED
LIVERPOOL SCHOOL OF TROPICAL MEDICINE

FOUNDED BY SIR ALFRED LEWIS JONES, K.C.M.G.

(Affiliated with the University of Liverpool)

Patron : HIS MAJESTY THE KING

Hon. President :

Hon. Vice-Presidents : THE EARL OF DERBY, K.G., G.C.V.O., C.B., LL.D.
SIR EDWARD MEREWETHER, K.C.V.O.
SIR H. J. READ, K.C.M.G.

Chairman : THE VISCOUNT LEVERHULME

Vice-Chairman : Mr. H. D. DICKIE

COUNCIL

THE VICE-CHANCELLOR OF THE UNIVERSITY OF LIVERPOOL — *University of Liverpool*

THE VISCOUNT LEVERHULME
Professor H. E. ROAF, M.D., D.Sc., L.R.C.P., M.R.C.S. — *Council of University of Liverpool*

Professor W. H. WOOD, B.Sc., M.D.
Professor H. D. WRIGHT, B.A., M.D., D.Sc., M.R.C.P.E. — *Senate of University of Liverpool*

Mr. F. G. HEISE
Mr. PICTON H. JONES — *Liverpool Steamship Owners' Association*

Mr. J. N. SELLERS, *Shipowners' Association*

Mr. T. EDWARD LESCHER, O.B.E.
Mr. ARTHUR D. DEAN — *Liverpool Chamber of Commerce*

Professor D. B. BLACKLOCK, M.D.
Mr. G. BROCKLEHURST
Mr. J. R. DANSON, M.C.
Professor R. M. GORDON, M.D.
Sir RICHARD D. HOLT, Bart., J.P.
Mr. ROBERT L. HOLT
Mr. J. PICKERING JONES
Mrs. PERCY F. KIPLING
Sir JOHN NICHOLSON, Bart.
Sir ROBERT RANKIN, Bart., M.P.
Dr. T. SOUTHWELL, D.Sc., Ph.D., F.R.S.E.
Professor W. YORKE, M.D., F.R.C.P., F.R.S.

Mr. J. R. HOBHOUSE, M.C., *Hon. Treasurer*

Mr. J. L. MCCARTHY, F.C.I.S., *Hon. Secretary*, The Chamber of Commerce, 1, Old Hall Street, Liverpool, 3.

STAFF

TROPICAL MEDICINE :

Alfred Jones Professor of Tropical Medicine :
WARRINGTON YORKE, M.D., F.R.C.P., F.R.S.

Lecturer in Tropical Medicine :
A. R. D. ADAMS, M.D., D.T.M.

Tropical Surgery :
PROFESSOR R. E. KELLY, C.B., F.R.C.S.

Tropical Dermatology :
R. STOPFORD-TAYLOR, D.S.O., M.B., Ch.B., F.R.C.S.E.

Tropical Ophthalmology :
A. McKIE REID, M.C., M.B., Ch.B., F.R.C.S.

TROPICAL HYGIENE :

Professor of Tropical Hygiene :
D. B. BLACKLOCK, M.D., D.P.H.

Practical Sanitation :
PROFESSOR W. M. FRAZER, M.D., D.P.H.

Public Health Chemistry :
ASSOCIATE PROFESSOR W. H. ROBERTS, M.Sc., F.I.C.

Sanitary Engineering :
PROFESSOR R. G. BATSON, A.K.C., M.Inst.C.E., M.I.Mech.E.

Lecturer in Vital Statistics :
C. O. STALLYBRASS, M.D., D.P.H.

ENTOMOLOGY :

Dutton Memorial Professor of Entomology :
R. M. GORDON, M.D., Sc.D., M.R.C.P., D.P.H.

Assistant Lecturer and Demonstrator in Entomology :
D. S. BERTRAM, B.Sc.

PARASITOLOGY :

Lecturer in Charge of the Department :
T. SOUTHWELL, D.Sc., Ph.D., F.R.S.E.

Assistant Lecturer in Parasitology :
A. KIRSHNER, M.B., Ch.B., D.T.M.

Honorary Assistant Lecturer in Veterinary Parasitology :
G. O. DAVIES, M.V.Sc., M.R.C.V.S., D.V.H.

BACTERIOLOGY :

Professor of Bacteriology :
H. D. WRIGHT, B.A., M.D., D.Sc., M.R.C.P.E.

RESEARCH FELLOWSHIPS :

Caton Memorial Fellow :
Vacant.

Chemotherapy : Medical Research Council Research Workers :
E. M. LOURIE, M.B., B.S., D.P.H., D.T.M. & H.
J. DEVINE, B.Sc., Ph.D.

Junior Tropical Medical Research Fellow, Medical Research Council :
J. L. DALES, M.B., Ch.B.

MUSEUM :

Curator :
MARY G. BLACKLOCK, B.Sc., M.B., Ch.B., B.A.O., D.T.M.

LIBRARY :

Librarian :
GLADYS PHILLIPS, M.A.

ROYAL INFIRMARY : TROPICAL DEPARTMENT :

Physician :
PROFESSOR WARRINGTON YORKE, M.D., F.R.C.P., F.R.S.

Assistant Physician :
A. R. D. ADAMS, M.D., D.T.M.

Consulting Surgeon :
PROFESSOR R. E. KELLY, C.B., F.R.C.S.

Consulting Dermatologist :
R. STOPFORD-TAYLOR, D.S.O., M.B., Ch.B., F.R.C.S.E.

Consulting Ophthalmologist :
A. McKIE REID, M.C., M.B., Ch.B., F.R.C.S.

SIERRA LEONE RESEARCH LABORATORY :

Professor of Tropical Diseases of Africa :

Assistant Director :
T. H. DAVEY, M.D., D.T.M.

Leverhulme Research Assistant :
A. J. WALKER, M.D., C.M., D.T.M.

Junior Tropical Medical Research Fellow, Medical Research Council :
G. B. LUDLAM, M.B., Ch.B., D.T.M., D.T.H.

THE MARY KINGSLEY MEDAL

This medal was struck in commemoration of the work of the late Miss Mary Kingsley in West Africa, and is conferred in recognition of distinguished scientific achievement.

HONORARY RECIPIENTS

Her Royal Highness Princess Christian
Lord Lister
The Right Hon. Joseph Chamberlain
Prince Auguste d'Arenberg
Mrs. Pinnock
William Adamson
William Carter
Miss A. R. Caton
Mrs. John Middlemass Hunt

RECIPIENTS

1905—

Colonel Sir David Bruce
Robert Koch
A. Laveran
Sir Patrick Manson

1907—

B. Danilewsky
Charles Finlay
W. M. Haffkine
Camillo Golgi
Surgeon-General W. C. Gorgas
Theobald Smith

1910—

Sir William Macgregor
R. Blanchard
Anton Breinl
Angelo Celli
C. W. Daniels
Surgeon-General Sir Alfred Keogh
Colonel W. G. King
B. Nocht
G. H. F. Nuttall
Major Leonard Rogers
J. L. Todd
Surgeon-General Walter Wyman

1913—

Frederick V. Theobald

1917—

Griffith Evans

1919—

J. W. Scott Macfie
The Oswaldo Cruz Institute, Rio de Janeiro

1920—

Major E. E. Austen
Sir Arthur G. Bagshawe
Sir Andrew Balfour
A. L. G. Broden
Mrs. Chalmers, in recognition of the work of the late Dr. A. J. Chalmers
G. Grassi
R. T. Leiper
F. Mesnil
Edmond Sergent
C. W. Stiles
T. Zammit

1929—

G. Carmichael Low
G. A. K. Marshall
Robert Newstead
A. T. Stanton
J. W. W. Stephens
C. M. Wenyon

1934—

Henry Beeuwkes
Sir George Seaton Buchanan
Sir S. Rickard Christophers
Sir Malcolm Watson

THE ALAN H. MILNE MEDAL

This medal was struck to commemorate the late Alan H. Milne, C.M.G., the first Honorary Secretary of the School (1899-1917), and is awarded twice yearly on the recommendation of the examiners for the Diploma in Tropical Medicine.

RECIPIENTS

1921—
George Phillip Farmer Allen

1922—
Quintin Stewart

1923—
John Cecil Cruickshank

1924—
George Maclean
Frederick John Carlyle Johnstone
Bernard Langridge Davis

1925—
Khiwaja Samad Shah
Alfred Robert Davies Adams
Alfred J. Hawe

1926—
John McPhail Campbell
Triloki Nath Varma

1927—
Alexander M. Gillespie
Joseph Hector Pottinger
Ragade Sanjiva Rao

1928—
Joseph Fine

1929—
Robert Erskine Anderson
Aubrey Vernon Greaves
Ian Cameron Middleton

1930—
Thomas Herbert Davey
Richard Green
Thomas Wilson

1931—
Kenneth Tole Moir
Hugh James Heaven Spreadbury

1932—
Bhavani Shankar Rao Nirodi

1933—
Geoffrey Donald Lehmann

1934—
James Ross Innes
Aubrey Alleyne Gibbons

1935—
Herbert Raymond Shone
Abe Kirshner
Richard Joseph O'Connor

1936—
Rash Gour Ghoshal
Tanjore Somosundaram Subramaniam

1937—
Gilbert Bowman Ludlam
Mohib Ullah Khan

1938—
James Kay

CATON MEMORIAL FELLOWSHIP

This fellowship was founded in 1929 to commemorate the late Dr. Richard Caton, one of the original members of the governing body of the School, and for many years its Vice-Chairman.

HOLDERS

1930-33
Frank Hawking, M.D.

1933-34
Helen M. Russell, M.D., F.R.C.P.E.

1935
Geoffrey Richard Walker, M.B., Ch.B., D.T.M.

1935-36
Richard Joseph O'Connor, M.B., B.S., D.T.M.

NOTICE

The following courses of instruction are given by the Liverpool School of Tropical Medicine each year :—

(1) Two courses for the Diploma in Tropical Medicine, commencing on the 29th September, 1938, and the 5th January, 1939. The D.T.M. examinations are held in December, March and June.

(2) Two courses for the Diploma in Tropical Hygiene, commencing on the 21st April, 1938, and the 10th January, 1939. The D.T.H. examinations are held in March and June.

DIPLOMA IN TROPICAL MEDICINE

This Diploma shall be awarded only to candidates who possess a qualification to practise Medicine recognised for this purpose by the University, and who present satisfactory certificates of having attended approved courses of study, and pass the prescribed examination.

DIPLOMA IN TROPICAL HYGIENE

This Diploma can only be taken by those who have already obtained the D.T.M. of the University of Liverpool.

FEES

D.T.M. Course	Twenty Guineas
D.T.H. Course	Ten Guineas
Special Courses in Parasitology and Entomology	Fifteen Guineas
Diploma Examination	Five Guineas

Fee for use of a School microscope during one term .. One Guinea.

For prospectus and further information, application should be made to the Laboratory Secretary, School of Tropical Medicine, Pembroke Place, Liverpool, 3.

The following have obtained the Diploma in Tropical Medicine of the University of Liverpool :—

Diploma in Tropical Medicine

Date of Diploma	
1904	Augustine, Henry Joshua
1904	Bennett, Arthur King
1904	Bruce, William James
1904	Byrne, John Scott
1904	Clayton, Thomas Morrison
1904	Dalziel, John McEwen
1904	Dee, Peter
1904	Greenidge, Oliver Campbell
1904	Hehir, Patrick
1904	Khan, Saiduzzafor
1904	Laurie, Robert
1904	Maclurkin, Alfred Robert
1904	McConnell, Robert Ernest
1904	Nicholson, James Edward
1904	Philipson, Nicholas
1904	Sharman, Eric Harding
1904	Thomson, Frank Wyville
1904	Walker, George Francis Clegg
1905	Anderson, Catherine Elmslie
1905	Brown, Alexander
1905	Caldwell, Thomas Cathcart
1905	Critien, Attilio
1905	Hooton, Alfred
1905	Hudson, Charles Tilson
1905	Illington, Edmund Moritz
1905	Macfarlane, Robert Maxwell
1905	Maddock, Edward Cecil Gordon
1905	Moore, James Jackson
1905	Nightingale, Samuel Shore
1905	Radcliffe, Percy Alexander Hurst
1905	Young, John Cameron
1906	Adie, Joseph Rosamond
1906	Arnold, Frank Arthur
1906	Bate, John Brabant
1906	Bennetts, Harold Graves
1906	Carter, Robert Markham
1906	Chisholm, James Alexander
1906	Clements, Robert William
1906	Dundas, James
1906	Faichnie, Norman
1906	Jeffreys, Herbert Castelman
1906	Mackenzie, Donald Francis
1906	Pailthorpe, Mary Elizabeth
1906	Palmer, Harold Thornbury
1906	Pearse, Albert
1906	Sampey, Alexander William
1906	Smithson, Arthur Ernest
1906	Taylor, Joseph van Someron
1906	Taylor, William Irwin
1906	Tynan, Edward Joseph
1906	Watson, Cecil Francis
1906	Willcocks, Roger Durant
1906	Williamson, George Alexander
1907	Allan, Alexander Smith
1907	Allwood, James Aldred
1907	Bond, Ashton
1907	Branch, Stanley
1907	Collinson, Walter Julius
1907	Davey, John Bernard
1907	Donaldson, Anson Scott
1907	Fell, Matthew Henry Gregson
1907	Gann, Thomas William Francis
1907	Graham, James Drummond
1907	Hiscock, Robert Carroll
1907	Keane, Joseph Gerald
1907	Kennan, Richard Henry
1907	Kenrick, William Hamilton
1907	Le Fanu, George Ernest Hugh
1907	Mackey, Charles
1907	Maddox, Ralph Henry
1907	McCarthy, John McDonald
1907	Raikes, Cuthbert Taunton
1907	Ryan, Joseph Charles
1907	Vallance, Hugh
1908	Caverhill, Austin Mack
1908	Crawford, Gilbert Stewart
1908	Dalal, Kaikhusroo Rustomji
1908	Dansey-Browning, George
1908	Davidson, James
1908	Dickson, John Rhodes
1908	Dowdall, Arthur Melville
1908	Glover, Henry Joseph
1908	Greaves, Francis Wood
1908	Goodbody, Cecil Maurice
1908	Harrison, James Herbert Hugh
1908	Joshi, Lemuel Lucas
1908	Le Fanu, Cecil Vivian
1908	Luethgen, Carl Wilhelm Ludwig
1908	Mama, Jamshed Byramji
1908	McCay, Frederick William
1908	McLellan, Samuel Wilson
1908	Pearce, Charles Ross
1908	Schoorel, Alexander Frederik
1908	Smith, John Macgregor
1908	Stewart, George Edward
1908	Tate, Gerald William
1908	Whyte, Robert
1909	Abercrombie, Rudolph George
1909	Allin, John Richard Percy
1909	Armstrong, Edward Randolph
1909	Barrow, Harold Percy Waller
1909	Beatty, Guy
1909	Carr-White, Percy
1909	Chevallier, Claude Lionel
1909	Clark, William Scott
1909	Cope, Ricardo
1909	Fleming, William
1909	Hanschell, Hother McCormick
1909	Hayward, William Davey
1909	Henry, Sydney Alexander
1909	Innes, Francis Alexander
1909	Jackson, Arthur Frame
1909	Kaka, Sorabji Manekji
1909	McCabe-Dallas, Alfred Alexander Donald
1909	Meldrum, William Percy
1909	Murphy, John Cullinan
1909	Samuel, Mysore Gnananandaraju
1909	Shroff, Kawasjee Byramjee
1909	Thornely, Michael Harris
1909	Turkhud, Violet Ackroyd

Date of Diploma	
1909	Webb, William Spinks
1909	Yen, Fu-Chun
1910	Brabazon, Edward
1910	Castellino, Louis
1910	Caulcrick, James Akilade
1910	Dowden, Richard
1910	Haigh, William Edwin
1910	Hamilton, Henry Fleming
1910	Hefferman, William St. Michael
1910	Hipwell, Abraham
1910	Homer, Jonathan
1910	Houston, William Mitchell
1910	James, William Robert Wallace
1910	Johnstone, David Patrick
1910	Korke, Vishnu Tatyaji
1910	Macdonald, Angus Graham
1910	Macfie, John Wm. Scott
1910	Manuk, Mack Walter
1910	Murison, Cecil Charles
1910	Nanavati, Kishavlal Balabha
1910	Nauss, Ralph Welty
1910	Oakley, Philip Douglas
1910	Pratt, Ishmael Charles
1910	Sabastian, Thiruchelvam
1910	Shaw, Hugh Thomas
1910	Sieger, Edward Louis
1910	Sousa, Pascal John de
1910	Souza, Antonio Bernardo de
1910	Waterhouse, John Howard
1910	White, Maurice Forbes
1911	Blacklock, Donald Breadalbane
1911	Brown, Frederick Forrest
1911	Chand, Diwan Jai
1911	Holmes, John Morgan
1911	Ievers, Charles Langley
1911	Iles, Charles Cochrane
1911	Ingram, Alexander
1911	Kirkwood, Thomas
1911	Knowles, Benjamin
1911	Liddle, George Marcus Berkeley
1911	Lomas, Emanuel Kenworthy
1911	Mackarell, William Wright
1911	MacKnight, Dundas Simpson
1911	Mascarenhas, Joseph Victor
1911	Murray, Ronald Roderick
1911	Oluwole, Akidiya Ladapo
1911	Rao, Koka Ahobala
1911	Sinton, John Alexander
1911	Tarapurvalla, Byramji Shavakshah
1911	Taylor, John Archibald
1911	Woods, William Medlicott
1912	Aeria, Joseph Reginald
1912	Anderson, Edmund Litchfield
1912	Borle, James
1912	Bowie, John Tait
1912	Brassey, Laurence Percival
1912	Christie, David
1912	Dillon, Henry de Courcy
1912	Dunn, Lillie Eleanor
1912	Hardwicke, Charles
1912	Jagose, Jamshed Rustomji
1912	Kochhar, Mela Ram
1912	McGusty, Victor William Tighe
1912	Milne, Arthur James
1912	Mitra, Manmatha Nath
1912	Myles, Charles Duncan
1912	Pelly, Huntly Nevins
1912	Prasad, Bindeshwari
1912	Prentice, George
1912	Ross, Frank
1912	Russell, Alexander James Hutchinson
1912	Ruthven, Morton Wood
1912	Sandilands, John
1912	Seddon, Harold
1912	Smalley, James
1912	Strickland, Percy Charles Hutchison
1912	Watson, William Russel
1913	Austin, Charles Miller
1913	Banker, Shiavux Sorabji
1913	Becker, Johann Gerhardus
1913	Carrasco, Milton
1913	Clark, James McKillican
1913	Forsyth, Charles
1913	Grahame, Malcolm Claude Russell
1913	Grieve, Kelburne King
1913	Hargreaves, Alfred Ridley
1913	Hepper, Evelyn Charles
1913	Hiranand Pandit
1913	Jackson, Oswald Egbert
1913	Khaw, Ignatius Oo Kek
1913	MacKelvie, Maxwell
1913	MacKinnon, John MacPhail
1913	Macmillan, Robert James Alan
1913	Mouat-Biggs, Charles Edward Forbes
1913	Noronha, John Carmel
1913	O'Connor, Edward
1913	Olubomi-Beckley, Emanuel
1913	Pestonji, Ardeshir Behramshah
1913	Puttanna, Dodballapur Sivappa
1913	Reford, John Hope
1913	Smith, Edward Arthur
1913	Stewart, Samuel Dudley
1913	Walker, Frederick Dearden
1913	Wilbe, Ernest Edward
1913	Wilson, Hubert Francis
1913	Yin, Ulg Ba
1913	Young, William Alexander
1914	Arculli, Hassan el
1914	Chohan, Noormahomed Kasembha
1914	Connell, Harry Bertram
1914	Gerrard, Herbert Shaw
1914	Gimi, Hirji Dorabji
1914	Gwynne, Joseph Robert
1914	Hodkinson, Samuel Paterson
1914	Jackson, Arthur Ivan
1914	Kaushash, Ram Chander
1914	Kelsall, Charles
1914	Luanco y Cuenca, Maximino
1914	Misbah, Abdul-Ghani Naguib
1914	Naidu, Bangalore Pasupulati Balakrishna
1914	Rowe, John Joseph Stephen
1914	Roy, Raghu Nath
1914	Shiveshwarkar, Ramchandra Vishnu
1914	Sur, Sachindra Nath
1914	Talati, Dadabhai Cursedji
1914	Wilkinson, Arthur Geden
1914	Wright, Ernest Jenner
1915	Lobo, John Francis
1915	Madhok, Gopal Dass
1915	Pearson, George Howorth
1915	Swami, Karumuri Virabhadra
1915	Wood, John

Date of Diploma	
1916	Barseghian, Mesroob
1916	Chaliha, Lakshmi Prasad
1916	Lim, Albert Liat Juay
1916	Lim, Harold Liat Hin
1916	Metzger, George Nathanie
1916	Söderström, Erik Daniel
1916	Wheeler, Louis
1917	Chapman, Herbert Owen
1917	Krishnamoorthy, Yedatore Venkoba
1917	Lipkin, Isaac Jacob
1918	Watts, Rattan Claud
1919	Bowle-Evans, Charles Harford
1919	Burnie, Robert McColl
1919	Celestin, Louis Abel
1919	Cummings, Eustace Henry Taylor
1919	Darling, Georgina Renington
1919	Drake, Joan Margaret Fraser
1919	Fraser, William James
1919	Gordon, Rupert Montgomery
1919	Krige, Christian Frederik
1919	Maplestone, Philip Alan
1919	Oluwole, Isaac Ladipo
1919	Rustomjee, Khusshuyee Jamesidjee
1919	Sawers, William Campbell
1919	Thompson, Mary Georgina
1919	Turner, Gladys Maude
1919	Young, Charles James
1920	Adler, Saul
1920	Anderson, William Jenkins Webb
1920	Campbell, George
1920	Cobb, Charles Eric
1920	Cobb, Enid Margaret Mary
1920	Connolly, Evelyn Mary
1920	Fernandez, Daniel David
1920	Lim, Chong Eang
1920	McHutchison, George Browne
1920	van der Merwe, Frederick
1920	O'Farrell, Patrick Theodore Joseph
1920	Renner, Edowo Awunor
1920	Vaughan, James Churchwill
1920	Waller, Harold William Leslie
1921	Allen, George Phillip Farmer
1921	Corfield, Charles Russell
1921	Hamid, Abdul
1921	Longhurst, Bell Wilmott
1921	Macvae, George Anthony
1921	Madan, Hans Raj
1921	Mulligan, William Percival
1921	Nixon, Robert
1921	Richmond, Arthur Stanley
1921	Shri Kent, Shamsher Singh
1921	Skinner, James Macgregor
1921	Stewart, Robert Bell
1921	Thomson, Marion
1922	Bhatia, Jagat Ram
1922	Cohen, Morris Joshua
1922	Crawford, Andrew Clemmey
1922	Gilmore, Edward Raymond
1922	Gracias, Cajetan Manuel
1922	Jennings, Arthur Richard
1922	Lethem, William Ashley
1922	Paul, Sachchidananda Hoshen
1922	Pinder, John

Date of Diploma	
1922	Rieley, Stanley Desmond
1922	Rutherford, Gladys
1922	Stewart, Quintin
1923	Abelman, B.
1923	Basu, Dhirendranath
1923	Cruickshank, John Cecil
1923	Doherty, Winifred Irene
1923	Edghill, Winifred M.
1923	Elsohn, John
1923	Fraser, N. D.
1923	Lee, R.
1923	Peirce, E. R.
1923	Raja, Rojaporum
1923	Reid, C. B. B.
1923	Richmond, A. E.
1923	Steven, J. B.
1923	White, Charles Francis
1924	Bilimoria, H. S.
1924	Carson, J. C.
1924	Chopra, B. L.
1924	Davis, B. L.
1924	Hardy, M. J.
1924	Jennings, C. B.
1924	Johnstone, F. J. C.
1924	Keirans, J. J.
1924	Lee, S. W. T
1924	Macdonald, G.
1924	Maclean, G.
1924	Mathur, W. C.
1924	Mitchell, J. M.
1924	Owen, D. Uvedale
1924	Palmer-Jones, Beryl
1924	Sankeralli, E. J.
1924	Singh, H.
1924	Theron, Elizabeth M.
1925	Adams, Alfred Robert Davies
1925	Ashton, Frank Richard
1925	Ashworth, Esther
1925	Bamford, Charles Walker
1925	Beinashowitz, Jack
1925	Black, John
1925	Clark, George
1925	Coghlan, Bernard A.
1925	Collier, Ivy
1925	Crawford, E. J.
1925	Cumming, Patrick Grant
1925	Ellam, Mary Muriel
1925	Fisher, Morris
1925	Green, Frederick Norman
1925	Grutu, M. S.
1925	Hawe, Albert J.
1925	Jafri, Z. H.
1925	Johnstone, Elvy I.
1925	Kerr, James R.
1925	Mackay, Donald M.
1925	Mackay, E. K.
1925	Makkawi, M.
1925	Maldonado, Leopoldo Garcia
1925	Mar, Severo Francisco
1925	Mozoomdar, B. P.
1925	Shah, Khwaja Samad
1925	Skan, Douglas A.
1925	Stone, Ernest R.
1925	Terrel, C. G.

Date of Diploma	
1925	Thompson, C. H. B.
1925	Tooth, Frederick
1925	de Waal, Jacobus Johannes
1926	Aitken, W. J.
1926	Ashworth, A.
1926	Austin, T. A.
1926	Bansikar, R. N.
1926	Besson, W. W.
1926	Bligh-Peacock, R. N.
1926	Bolton, Effie G.
1926	Boodrie, E. H.
1926	Brito-Mutunayagam, M. A. B.
1926	Campbell, J. McP.
1926	Cullen, T.
1926	Davies, H. N.
1926	Dias, B. G. V.
1926	Doherty H. A. A.
1926	Don, E. G.
1926	Earl, J. C. St. G.
1926	Fletcher, Beatrice N.
1926	Fowler, H. P.
1926	Fowler, Isabella J.
1926	Hamilton, J.
1926	Hodgkinson, Katharine
1926	Jackson, R.
1926	Kamakaka, K. H.
1926	Kennedy, J. H.
1926	Khatri, L. D.
1926	Lennox, D.
1926	Lewis, A. W.
1926	McConn, C. F.
1926	Mackay, A. G.
1926	McLean, N.
1926	MacSweeney, M.
1926	Malhautra, K. L.
1926	Malik, S. B.
1926	Manuwa, S. L. A.
1926	Merchant, M. E.
1926	Mitchell, W. H.
1926	Molony, E. F.
1926	Nashikkar, S. G.
1926	Oppenheimer, F.
1926	Ormiston, W. S.
1926	Paterson, F. S.
1926	Patterson. F. L.
1926	Puri, V. V.
1926	Quigley, L. D.
1926	Robertson, A.
1926	Rodrigues, M.
1926	Sachdev A. S.
1926	Singh, B.
1926	Singh, J.
1926	Talib, S. A.
1926	Tan, C. L.
1926	Taylor, Catherine F.
1926	Turnbull, N. S.
1926	Turner, J. G. S.
1926	Vardya, B. K.
1926	Varma, T. N.
1926	Voigt, C.
1926	Wasti, S. N.
1927	Allen, C. P.
1927	Bahl, M. L.
1927	Barrowman, B.

Date of Diploma	
1927	Bawa, H. S.
1927	Bilimoria, J. D
1927	Burns, W. M.
1927	Daly, E. J.
1927	Dunlop, G. A.
1927	Dyream, V.
1927	Evans, R. R.
1927	Farid, M.
1927	Gillespie, A. M.
1927	Gunawardana, S. A
1927	Harkness, J.
1927	Hay, R.
1927	Hodivala, N. M.
1927	Hughes, Emma
1927	Hyslop, Kathleen M.
1927	Ingram-Johnson, R. E.
1927	Kapadia, J. S.
1927	Khan, F. A.
1927	Khan, M. M.
1927	Labuschagne, P. N. H.
1927	Laird, W. J.
1927	Lewin, B. F.
1927	Macdonald, J.
1927	McElroy, R. S.
1927	Maclay, W. S.
1927	Maguire, H. G.
1927	Mahaffy, A. F.
1927	Malhotra, A. L
1927	Malhotra. H. L.
1927	Manghirmalani, B. S.
1927	Meek, A. I.
1927	Mehra, J. N.
1927	Mehta, H. C.
1927	Menon, M. V.
1927	Miller, H. V. R.
1927	Mokand, S. N.
1927	Murgatroyd, F.
1927	Murray, A. J.
1927	Murray, Pauline V.
1927	Nevin, H. M.
1927	Nirula, P. N.
1927	Olusoga, N. T.
1927	Parakh, D. B.
1927	Peters, D. O.
1927	Peters, M. R.
1927	Pottinger J. H.
1927	Rao, R. S.
1927	Rodriguez, G. V. S.
1927	Shah, S. R. A.
1927	Singh, H.
1927	Southward, J. F.
1927	Sturton, S. D.
1927	Thompson, Frances C.
1927	de Villiers, B. J. van de S.
1927	Walkingshaw, R.
1927	Wilkinson, S. A.
1928	Ahluwalia, C. L.
1928	Aidin, A. R.
1928	Anand, J. S.
1928	Askari, S. W. H.
1928	Beveridge, Ruby S.
1928	Biswas, M. K.
1928	Blakemore, W. L.
1928	Camps-Campins, J. M.

Date of Diploma	
1928	Chacko, M. O.
1928	Chopra, A. N.
1928	Chaudhuri, J. P.
1928	Choudari, K. V. R.
1928	Cranage, Margaret
1928	Dhala, C. H.
1928	Dhar, K. K.
1928	Dikshit, H. K.
1928	Everard, N. J.
1928	Fine, J.
1928	Ghei, A. N.
1928	Halawani, A.
1928	Henshaw, L. E. R.
1928	Hilmy, I. S.
1928	Holmes, W. E.
1928	Hope-Gill, C. W.
1928	Kane, F.
1928	Katial, C. L.
1928	Khan, F. M.
1928	Krishna, R.
1928	Lawrence, H. S.
1928	Lawrence, M. R.
1928	McLaren, D. W.
1928	Malhotra, B. D.
1928	Mallick, B. D.
1928	Mason, Jean R.
1928	Menon, E. S. R.
1928	Milne, J.
1928	Mitchell, A.
1928	Mone, R. V.
1928	Morley, A. H.
1928	Mostert, H. van R.
1928	Mufty, S.
1928	van Niekerk, S. V.
1928	Pandit, M. K.
1928	Pearce, W. T. A.
1928	Plum, D.
1928	Rao, B. D.
1928	Reid, A.
1928	Sanderson, I.
1928	Setna, H. M.
1928	Shearer, G.
1928	Singh, B.
1928	Sivalingam, S.
1928	Stratton, Ella M.
1928	Suri, R.
1928	Tuli, R. L.
1928	Udvadia, F. F.
1928	Wagle, P. M.
1928	Wahid, A.
1928	Wall-Mesham, Nellie
1928	Whig, P. L.
1929	Ahuja, S. D.
1929	Anderson, R. E.
1929	Booker, C. G.
1929	Bullen, W. A.
1929	Callum, E. N.
1929	Chakravarti, K. B.
1929	Connolly, P. P. D.
1929	Cowan, J. A.
1929	Crawford, J.
1929	Dale, W. C.
1929	Dogra, J. R.
1929	Drury, G. D.

Date of Diploma	
1929	Gill, T. S.
1929	Graham-Cumming, G.
1929	Greaves, A. V.
1929	Hale, G. S.
1929	Herbertson, Margaret A. L.
1929	Howell, A. T.
1929	Innes, J. A. L.
1929	Latham, C. N.
1929	McGregor, J. A.
1929	McMahon, J. E.
1929	McQueen, W. B.
1929	Majumdar, B. K.
1929	Middleton, I. C.
1929	Miller, A. A.
1929	Pearse, J. T. F.
1929	Ramdeholl, C.
1929	Robinson, Elizabeth J.
1929	Robinson, P. B.
1929	Rosenbloom, A.
1929	Row, C. K.
1929	Sewal, R. N.
1929	Shafi, A.
1929	Singh, H.
1929	Talwrn-Jones, G. A.
1929	Turner, H. N.
1929	Verghese, T.
1929	Wilson, S. P.
1930	Bagchi, K. W.
1930	Baxter, G. R.
1930	Bier, L. B.
1930	Boyd, C. J.
1930	Brown, J. A. K.
1930	Caplan, J.
1930	Cathcart, J. A.
1930	Chen, T. T. M.
1930	Chowdhary, D. S.
1930	Davey, T. H.
1930	Deacon, Ariel R. S.
1930	Dobbin, J. H.
1930	Gillespie, F. D.
1930	Ghosh, L. M.
1930	Grant, S. C.
1930	Green, R.
1930	Gulatee, M. L.
1930	Hawking, F.
1930	Heatley, R. A.
1930	Khanna, B. N.
1930	Lindsay, D. K. L.
1930	Loewenthal, L. J. A.
1930	McElwee, D.
1930	MacKenzie, K. C.
1930	Mackie, J. B.
1930	Mangrulkar, Y. B.
1930	Mendis, J. E. D.
1930	Miau, W.-K.
1930	Mody, M. B.
1930	Mohile, G. B.
1930	Mulligan, H. W.
1930	Narain, S.
1930	O'Driscoll, F
1930	Poh, C. J.
1930	Singh, M.
1930	Sumitra, L.
1930	Sung, J. K.

Date of Diploma	
1930	Verghese, G.
1930	Webster, J. L. A.
1930	Willoughby, J.
1930	Wilson, T.
1931	Behari, R.
1931	Bhambari, K. L.
1931	Bhargava, G. S.
1931	Bowers, G. P. F.
1931	Camps, F. E.
1931	Carrol, A. E.
1931	Chandra, P.
1931	Chaturvedi, H. S.
1931	Craven, W. H.
1931	Crawford, W.
1931	Cutting, P. E. J.
1931	Fitzgerald, J. A. K.
1931	Gauld, E. R.
1931	Guha, A. K.
1931	Henry, A. F. X.
1931	Kalra, M.
1931	Kuo, J. T.
1931	Kuruvila, P. K.
1931	Lakhwarah, M.
1931	Low, E. W.
1931	Maass, E. W. H.
1931	McNair, H.
1931	Maniar, H. A.
1931	Mansur, J.
1931	Menon, V. C. G.
1931	Moir, K. T.
1931	Peaston, H.
1931	Salah El Din, M.
1931	Sardana, M. N.
1931	Sharma, D. R.
1931	Speirs, R. C.
1931	Spreadbury, H. J. H.
1931	Tarneja, L.
1931	Tripp, Dorothy M. H.
1931	Ullah, S.
1931	Yunibandhu, J.
1932	Adams, H. E. M.
1932	Bando, F.
1932	Bhandari, B. N.
1932	Bindra, B. S.
1932	Bowesman, R.
1932	Chan, C. T.
1932	Dass, P.
1932	Doherty, J. A.
1932	Haji, H. S.
1932	Ling, L. C.
1932	Lowe, Greta
1932	Nirodi, B. S. R.
1932	Onwu, S. E.
1932	Portway, R. L.
1932	Rainsford, C. R. C.
1932	Roseveare, Margaret P.
1932	Smith, R. B. S.
1932	Sun, T. C. Y.
1932	Tomb, J. S.
1932	Williams, E. G.
1932	Zau, F. D.
1933	Amirtha Nayagam, G. P. A.
1933	Anderson, N. E. W.
1933	Armah, J. E.

Date of Diploma	
1933	Athavale, V. B.
1933	Chan, L. F.
1933	Day, F. M.
1933	Din, N.
1933	Dyal, F.
1933	Feng, L. C.
1933	Ghosh, M. M.
1933	Greson, L. P.
1933	Gribbon, G. R.
1933	Hassouna, M. A. L
1933	Ismail, A. H.
1933	Jackson, S.
1933	Kuraishy, N. A.
1933	Lehmann, G. D.
1933	Luke, J. F.
1933	McTaggart, Christina M
1933	Mathur, R. B.
1933	Nanjiani, B. S.
1933	Robertson, J. D.
1933	Ross, C. M.
1933	Shaw, Freni M.
1933	Silva, C. W. A. de
1933	Thompson, E. F.
1933	Walker, G. R.
1933	Zan, M. S.
1934	Ahmed, A.
1934	Amzalak, E. S.
1934	Appleton, S. K.
1934	Arulpragasam, A. R
1934	Awoliyi, S. O.
1934	Beausang, J. J.
1934	Cantin, J. J. A.
1934	Chang, P. Y.
1934	Cooper, S. W.
1934	Cosgrove, P. C.
1934	Craddock, F. R.
1934	Dunlop, R. Y.
1934	Edwards, A. C.
1934	Foulds, T.
1934	Ghosh, I. H. B.
1934	Gibbons, A. A.
1934	Hamid, B. A.
1934	Hughes, W.
1934	Innes, J. R.
1934	Kallianpurkar, S. L.
1934	Ling, C. C.
1934	McElney, J. H.
1934	McMillan, J. S.
1934	Macpherson, D. C. M.
1934	Malone, T. A.
1934	Nyi, Z. Z.
1934	Purcell, P. J.
1934	Strangway, W. E.
1934	Tay, K. S.
1934	Vakil, Banoo V.
1934	Wu, T. T.
1935	Anderson, V. F.
1935	Board, A. J.
1935	Daniell, Alva A.
1935	Ezzat, H. F.
1935	Farooq, M.
1935	Hakeem, A. Z.
1935	Hamid, M. A.
1935	Harmer, Freda
1935	Kamel, A.

Date of Diploma	
1935	Khalil, M. A. M.
1935	Kirshner, A.
1935	Lahiri, M. N.
1935	Li, C. H.
1935	Mahallawy, A. H. S.
1935	Morton, Marjorie B.
1935	Nasr, M. A.
1935	O'Connor, R. J.
1935	O'Toole, K.
1935	Patel, R. V.
1935	Robertson, J. R.
1935	Shone, H. R.
1935	Strange, S. L.
1935	Symonds, J. W. C.
1935	Ting, S. K.
1935	el Tobgy, M. A.
1935	Tomlinson, Mary A.
1935	Tu, T. P.
1935	Walker, A. J.
1935	Weeks, E. B.
1936	Amegatcher, J. E. O.
1936	Barnetson, W.
1936	Bhaduri, D. N.
1936	Boules, A.
1936	Bradbury, E.
1936	Chakravarti, N.
1936	Cran, D. L.
1936	Easmon, R. S. A.
1936	Gandhi, N. G.
1936	Ghoshal, R. G.
1936	Gomaah, M. H.
1936	Green, B. J.
1936	Hazra, S.
1936	Hiremath, A. G.
1936	Ho, Chin Lien.
1936	Hurtado, L.
1936	Ismail, M. T.
1936	Mukerjee, B. B.
1936	Murray, W.
1936	Rao, M. S.
1936	Saleem, S.
1936	Sankaran, K.
1936	Slimon, J. G.
1936	Subramaniam, T. S.
1936	Summerhayes, J. W.
1936	Tabet, A.
1936	Wang, S.
1937	Bamford, P. V.
1937	Barrow, P. G.
1937	Casson, A. H.
1937	Clarke, P. E. M.
1937	Craine, G. V.
1937	Crook, F. W.
1937	Dales, J. L.
1937	Daley, E. A.
1937	Downes-Shaw, Alma
1937	Ghose, S. K.
1937	Hamid, M. S. B. A.
1937	Hutchinson, Roberta I.
1937	Khan, M. U.
1937	Kok, O. V. S.
1937	Kotak, C. H.
1937	Lake, F.
1937	Livera, P. L. F. de
1937	Luanprida, I.
1937	Ludlam, G. B.
1937	Mahadevan, M. N.
1937	Manwell, W.
1937	Mathew, K. C.
1937	Mazzotti, L.
1937	Patwardhan, V. G.
1937	Prasad, H.
1937	Robert, S. L.
1937	Siddique, Y. M.
1937	Singh, N. N.
1937	Syed, S. H. R.
1937	West, Elizabeth H.
1937	West, H. F.
1937	Wilson, J. D. T.
1937	Wislicki, Luise
1937	Yofé, J.
1938	Bastawros, F.
1938	Bourke, P. J.
1938	Einstein, O.
1938	Hesse, N. Q.
1938	Jacusiel, F.
1938	Jilari, K.
1938	Kay, J.
1938	Kohlschütter, E.
1938	Lanceley, J. L.
1938	Nyan, B.

The following have obtained the Diploma in Tropical Hygiene of the University of Liverpool :—

Diploma in Tropical Hygiene

Date of Diploma	
1926	Aitken, W. J.
1926	Bligh-Peacock, N.
1926	Clark, G.
1926	Collier, Ivy
1926	Cullen, T.
1926	Davis, B. L.
1926	Don, E. G. A.
1926	Fowler, H. P.
1926	Hawe, A. J.
1926	Lennox, D.
1926	Mackay, A. G.
1926	Mackay, D. M.
1926	McLean, N.
1926	MacSweeney, M.
1926	Oppenheimer, F.
1926	Skan, D. A.
1926	Talib, S. A.
1926	Turnbull, N. S.
1927	Allen, C. P.
1927	Austin, T. A.
1927	Besson, W. W.
1927	Dunlop, G. A.
1927	Earl, J. C. St. G.
1927	Hamilton, J.

Date of Diploma	
1927	Harkness, J.
1927	Hay, R.
1927	Hyslop, Kathleen M.
1927	Labuschagne, P. N. H.
1927	McCon, C. F.
1927	Macdonald, J.
1927	Mitchell, Winifred H.
1927	Murray, A. J.
1927	Nevin, H. M.
1927	Nixon, R.
1927	Ormiston, W. S.
1927	Robertson, A.
1927	Walkingshaw, R.
1928	Bilimoria, J. D.
1928	Blakemore, W. L.
1928	Choudari, K. V. R.
1928	Dhar, K. K.
1928	Evans, R. R.
1928	Holmes, W. E.
1928	Laird, W. F.
1928	Maclay, W. S.
1928	Miller, H. V. R.
1928	Morley, A. H.
1928	Pearson, G. H.
1928	Pottinger, J. H.
1928	Sanderson, I.
1928	Sivalingam, S.
1928	Wilkinson, S. A.
1929	Askari, S. W. H.
1929	Drury, G. D.
1929	Fraser, N. D.
1929	Halawani, A.
1929	Hilmy, I. S.
1929	Innes, J. A. L.
1929	Lawrence, H.
1929	Nixon, R.
1929	Ramdeholl, C.
1929	Setna, H. M.
1930	Anderson, R. E.
1930	Baxter, G. R.
1930	Booker, C. G.
1930	Bullen, W. A.
1930	Chen, T. T. M.
1930	Dobbin, J. H.
1930	Heatley, R. A.
1930	Krishna, R.
1930	Latham, C. N.
1930	McHutchison, G. B.
1930	McMahon, J. E.
1930	Menon, E. S. R.
1930	Reid, A.
1930	Row, C. K.
1930	Wilson, T.
1931	Camps, F. E.
1931	Gauld, E. R.
1931	Loewenthal, L. A.
1931	Mendis, J. E. D.
1931	Moir, K. T.

Date of Diploma	
1931	O'Driscoll, F.
1931	Salah, el Din, M.
1931	Sung, J. Koh-si
1931	Verghese, G.
1932	Bowers, G. P. F.
1932	Cathcart, A.
1932	Kurivila, T. K.
1932	Middleton, I. C.
1932	Spreadbury, H. J. H.
1933	Bando, F.
1933	Banker, S. S.
1933	Lowe, Greta
1933	Onwu, S. E.
1933	Rosenbloom, A.
1933	Williams, E. G.
1933	Zau, F. D.
1934	Anderson, N. E. W.
1934	Arulpragasam, A. R.
1934	Athavale, V. B.
1934	Feng, L. C.
1934	Ghosh, I. H. B.
1934	Greson, L. P.
1934	Ling, C. C.
1934	Malone, T. A.
1934	Manuwa, S. L. A.
1934	McMillan, J. S.
1934	Silva, C. W. A. de
1935	Awoliyi, S. O.
1935	Cooper, S. W.
1935	Cosgrove, P. C.
1935	Dunlop, R. Y.
1935	Mackenzie, K. C.
1935	Nayagam, G. P. A.
1935	Nyi, Z. Z.
1935	Shone, H. R.
1936	Barnetson, W.
1936	Cran, D. L.
1936	Crawford, W.
1936	O'Toole, K.
1936	Smith, R. B. S.
1936	Ting, S. K.
1936	Tu, T. P.
1937	Amegatcher, J. E. O.
1937	Bradbury, E
1937	Gomaah, M. H.
1937	Green, B. J.
1937	Hazra, S.
1937	Ludlam, G. B.
1937	Robertson, J. D.
1937	Saleem, S.
1937	Subramaniam, T S
1937	Summerhayes, J. W.
1937	Syed, S. H. R.
1937	Wislicki, Luise
1938	Dales, J. L.
1938	Patwardhan, V. G.

ANNALS OF TROPICAL MEDICINE AND PARASITOLOGY

EDITORIAL NOTICE

Articles for publication should not exceed twenty-five pages of the Annals, and will be understood to be offered alone to this Journal. They should be typewritten and addressed to :—The Editors, School of Tropical Medicine, Pembroke Place, Liverpool, 3.

Illustrations for text-figures or charts should be drawn clearly and firmly in Indian ink, if possible on Bristol board. N.B.—*Blue or other coloured* ruling in squares or lines cannot be reproduced.

All lettering, names or legends on text-figures, charts or maps should be printed *sufficiently large* to allow of *clear legibility* on reduction if necessary.

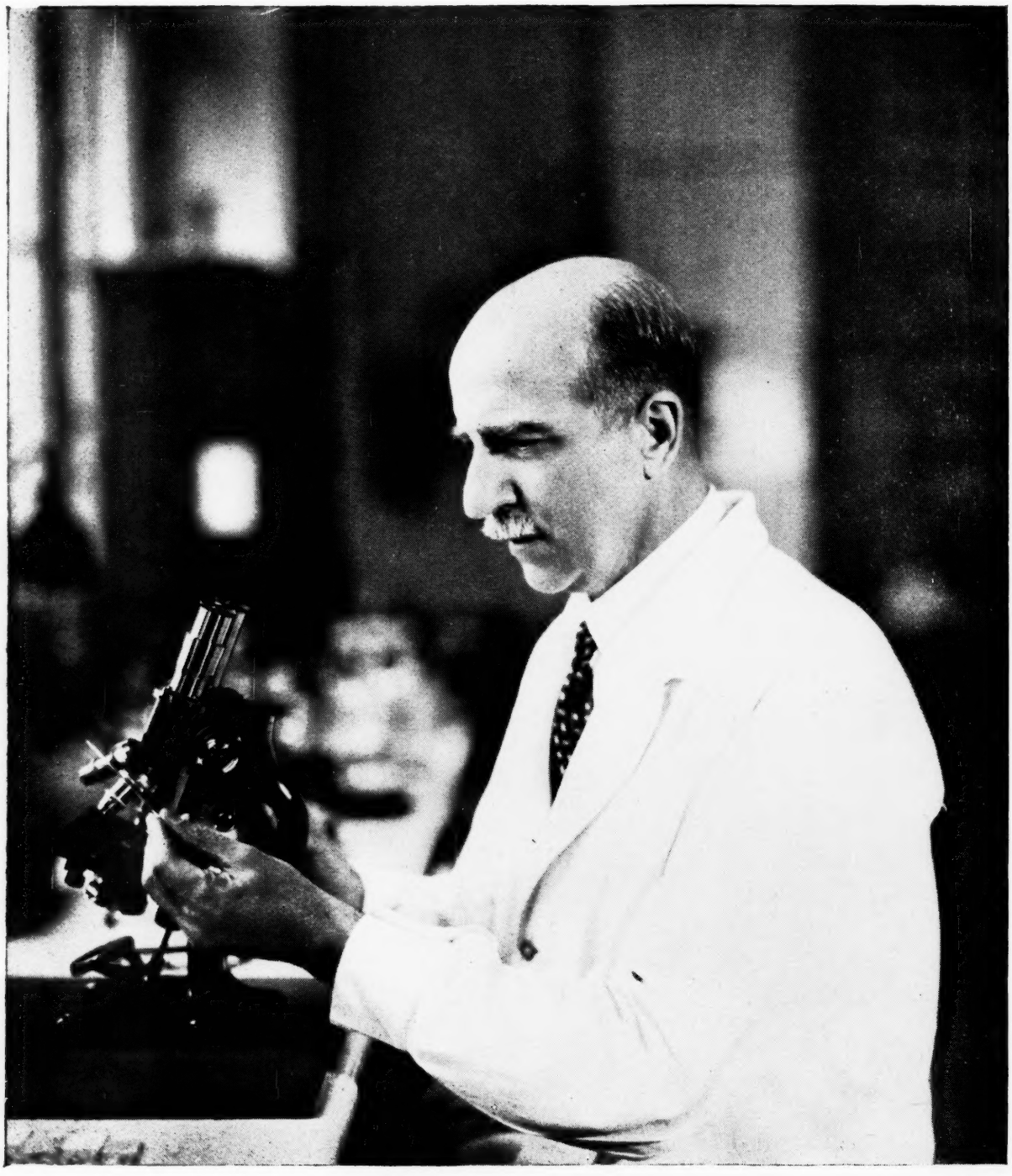
Plates and illustrations should be accompanied by short explanations.

References to authors in the text must be made in the following way :—' According to Smith (1900) the spleen is enlarged, but Robinson (1914) says the reverse.' The references should be collected in alphabetical order of authors' surnames at the end of the paper, and arranged in the following way :—

ROBINSON, S. (1914). The spleen in malaria. *Ann. Nosology*, **20**, 20.
SMITH, J. (1900). Enlargement of the spleen in malaria. *Jl. Pathometry*, **1**, 1.

Twenty-five reprints of each paper are supplied, free of charge. A further 25 reprints can be obtained at cost price. No further copies can be supplied.

Subscription : £1 2s. 6d. per volume, post free, payable in advance to The University Press of Liverpool, 177 Brownlow Hill, Liverpool, 3, to whom correspondence concerning advertisements should also be addressed.



UNIV. OF MICH.

LEAGUE OF NATIONS HEALTH ORGANISATION

TREATMENT OF MALARIA: STUDY OF THE THERAPEUTICS AND PROPHYLAXIS OF MALARIA BY SYNTHETIC DRUGS AS COMPARED WITH QUININE

FOURTH GENERAL REPORT OF THE MALARIA COMMISSION

(*Received for publication December 23rd*, 1937)

It will be remembered that in 1933 the Malaria Commission of the League of Nations published its third general report entitled ' The Therapeutics of Malaria.' This report was based chiefly on the knowledge acquired by a study of experimental malaria.

Two years later the Commission arranged for experiments to be made on the treatment and prophylaxis of malaria with synthetic drugs and with quinine so as to compare their efficacy. These experiments were conducted according to a plan which had been very carefully prepared beforehand and which involved the use of the same drugs, the same doses and the same technique. They covered 12,288 subjects and were carried out in Algeria, Italy, the Federated Malay States, Roumania and the U.S.S.R., under the direction of Professor Edmond Sergent, Professor Bastianelli, Dr. Neave Kingsbury, Professor Ciuca and Professor Serguieff, respectively.

The Commission has now drawn up the text of its fourth general report on the basis of the lessons to be learnt from these experiments, whilst paying due regard at the same time to the scientific papers that have been otherwise published on the question of the treatment of malaria. This report is the work of a drafting committee consisting of Professor Edmond Sergent, Chairman, Dr. Balfour, Professor Pittaluga and Colonel Sinton. It represents the unanimous views of the Commission. It is entitled ' The Treatment of Malaria ' and recently appeared in the *Bulletin of the Health Organisation* (1937). The annexes include an account of the experiments, a bibliographical review and the text of the observations made by the various members of the Malaria Commission.

We have pleasure in giving below the conclusions of the report.

I. ACTION OF QUININE AND OF SYNTHETIC PRODUCTS ON THE DIFFERENT MANIFESTATIONS OF MALARIAL INFECTION

(*a*) QUININE

1. *Action on the trophoxoites in primary infections.* A minimum daily dose of 0·50 gm. of quinine hydrochloride sometimes suffices to cause a temporary disappearance of the trophozoites of *P. vivax* ; but a *mean daily dosage* of 1 gm.

for 5 to 7 days is often necessary to cause the trophozoites to disappear (on an average on the third day) and not to make their reappearance in the peripheral blood until after a latent period of varying length, in the course of the first relapse. In quartan (*P. malariae*) the same effects are usually obtained. In infections with *P. falciparum*, the average effective daily dose should be fixed at about 1·30 gm. to produce analogous results. In some countries, it is even necessary to use 2 gm. in order to obtain a rapid effect upon the clinical attack and on the parasites. With the usual dose of 1 gm. the trophozoites generally disappear *1 day later*, on the average, than in the case of *P. vivax* ; sometimes their resistance continues even longer.

2. *Action on the gametocytes* of *P. vivax* and *P. malariae*. Quinine, in the doses indicated, exercises its parasiticidal activities on the young forms of *P. vivax* and *P. malariae* capable of producing gametocytes, and also on fully developed gametocytes. On the fully developed gametocytes of *P. falciparum*, quinine has only a very slight action ; but it also impedes the formation of the pre-gametocytes of this species. It may thus be regarded as directly schizonticidal and indirectly gametocidal in the case of *P. falciparum*.

3. *On the acute clinical symptoms of primary infection*, quinine, in the indicated doses, has a definite action from the third day onwards (second paroxysms of fever) in benign tertian ; its action is less reliable or less rapid, according to the strain of *P. falciparum* concerned, on attacks of malignant tertian, which often continue until the fifth dose (third or fourth paroxysm).

4. *On the frequency of relapses in general*, quinine has a clearly marked effect, which is, however, influenced by individual factors and by the strain of parasite. The treatment of primary *P. vivax* or *P. malariae* infections with quinine in the usual doses (1 gm. daily) is followed by relapses in a proportion of individuals which may be as high as 50 per cent.

5. *The action of quinine on splenomegaly*, when suitable treatment is applied in each attack, has proved to be of real efficacy in endemic regions, especially among children. It is but transient, however, if the community concerned is subject to a high proportion of relapses or is exposed to frequent reinfections.

6. Quinine treatment with the usual doses does not affect the patient's general condition adversely and generally has no depressive or toxic effect, if the period of administration is limited to the strictly necessary number of days. In such a case, there is no good reason for thinking that this treatment hinders the processes of immunization, but ill effects may occur when treatment is unnecessarily protracted.

(*b*) Atebrin

1. *Action on the trophozoites.* Atebrin in daily doses of 0·30 gm. (for adults) has a slightly more rapid action on *P. vivax* trophozoites than quinine in the usual dose of 1 gm. The trophozoites disappear on an average after the third dose, and in some cases even after the second. This parasiticidal action

appears to continue for a longer period, in that the phase of latency of the disease (absence of clinical symptoms) is established more certainly and lasts somewhat longer after the end of treatment with atebrin than with quinine. On the trophozoites of *P. malariae*, the action of atebrin can be said to be of the same nature. On the trophozoites of *P. falciparum*, atebrin is equally in advance of quinine in certain cases ; but the differences between the strains of parasite prevent the drawing of uniform conclusions. The trophozoites of *P. falciparum* disappear from the peripheral blood after the fourth dose of atebrin in 90 per cent. of cases.

2. *The action of atebrin on the gametocytes* is of a similar nature to that of quinine ; it has no effect, from the point of view of devitalization, on the gametocytes of *P. falciparum*. But the action on gametocytes already present in the blood is perhaps slightly more marked than that of quinine, particularly as regards the gametocytes of *P. vivax* and *P. malariae*.

3. *The action on the clinical symptoms of an acute attack is very marked, both in benign tertian and in malignant tertian.* In some endemic regions, where there may possibly be special strains of *P. falciparum*, the therapeutic action of atebrin is even more energetic on malignant tertian than on benign tertian. But, in other cases, the contrary seems to be true. This is why some practitioners and malariologists in tropical countries prefer to use quinine during the first days of the acute attack and to continue with atebrin thereafter. In benign tertian the fever nearly always falls after the first three therapeutic doses of atebrin—that is to say, by the second attack. In malignant tertian the fever falls almost invariably by the third attack.

4. *The action of atebrin on relapses is slightly more effective than that of quinine*, especially in the case of benign tertian and of certain strains of malignant tertian.

5. *The spleen rate* in communities treated with atebrin seems to decrease somewhat more slowly than in communities treated with quinine, but the effects of the drug continue to be felt for a longer time during the observation period after the end of the treatment, the decrease in the percentage of enlarged spleens continues longer, and the return of the splenic index figures to their former high level occurs a little later.

6. *The action of atebrin on the general condition of patients* seems to be determined by factors which, after this form of treatment, are still not entirely known—that is to say, by the action of the drug on the organic defences in general and on the processes of immunization. The yellow coloration of the skin produced by atebrin is a disadvantage, especially during prolonged prophylactic treatments.

(*c*) PLASMOQUINE

1. *Action on the trophozoites.* The action of plasmoquine on the trophozoites of *P. falciparum* is almost nil. It acts to some extent on the trophozoites

of *P. vivax*, and especially on those of *P. malariae*. With small non-toxic doses of plasmoquine associated with the usual doses of quinine or atebrin, better results are sometimes obtained on the trophozoites of *P. vivax* and even of *P. falciparum*.

2. *Plasmoquine acts upon the gametocytes of the three species, but especially on those of P. falciparum*, which are practically unaffected either by quinine or by atebrin. In minimum doses of 0·02 gm., plasmoquine devitalizes the gametocytes of *P. falciparum*, and at the same time diminishes their numbers.

3. There is no advantage in using plasmoquine alone for the *treatment of the clinical symptoms* of an acute attack in any of the forms of malarial infection.

4. *Plasmoquine has a definite effect upon the frequency of relapses* of benign tertian or quartan. In association with quinine or atebrin, or administered after either of these two drugs, it is to a marked degree effective in preventing relapses in benign tertian (except, perhaps, in the case of a few particular strains) and in quartan, and appears similarly to reduce the frequency of malignant tertian relapses.

5. We do not possess sufficient data to assess the action of plasmoquine alone, used either therapeutically or prophylactically, *upon the state of the spleens in malarial communities*, for it is nearly always administered together with other drugs.

6. The small doses of *plasmoquine* (0·02 gm., for example) that are now being used, seem to have no serious depressing effect on the general state of the patient. That the prolonged use of plasmoquine may exert some influence on the neoformation of haemoglobin must not be overlooked.

(*d*) Quinine-Atebrin, Quinine-Plasmoquine and Atebrin-Plasmoquine Combinations

1. The few experimental observations that have been published give no indication that there is any advantage in combining quinine and atebrin together for purposes of treatment.* Further clinical research is required in order to determine the effects of these two drugs, when administered one after the other (usually quinine first and atebrin afterwards) in the treatment of acute attacks of certain kinds of infection, especially *P. falciparum*.

2. The combined use of quinine with plasmoquine produces less frequent and less intense toxic symptoms than that of atebrin with plasmoquine. The association properly so called (that is to say, the *simultaneous* use) of quinine and plasmoquine (for example, up to 0·02 gm. or even 0·03 gm. of plasmoquine daily for short treatments) therefore does not involve any particular contra-indications. Certain authors recommend, however, that, whenever possible, the two drugs be administered consecutively. For the treatment of *adult groups*

---

* Professor Rodhain, in a private communication, states that he has recently obtained beneficial effects by this method.

under observation (soldiers, workers) there is, however, no serious disadvantage to be feared from the simultaneous use of quinine and plasmoquine, which, moreover, shortens the duration of treatment.

The association of quinine with plasmoquine represents one of the most efficacious methods of treating *benign tertian* and *quartan* malaria. Treatment with average doses (1 gm. to 1·30 gm.) of quinine-*plus*-plasmoquine (even only 0·02 gm. to 0·03 gm. twice a week) greatly reduces (perhaps more than any other method) the number of relapses in benign tertian (except, as already indicated, in the case of certain strains) and in some cases also in *malignant tertian.*

3. *The simultaneous administration of atebrin and plasmoquine* appears to aggravate the toxicity of each. It is not to be advised, though it is understood to have been used without ill effects in certain communities of adult men. It should in any case not be adopted without medical supervision. Consecutive treatment with atebrin first and then with plasmoquine in suitable doses (0·30 gm. atebrin daily for 5 or 7 days, followed by 0·02 gm. plasmoquine daily for 5 days) has no appreciable influence either in reducing the proportion of trophozoites in the blood or on the clinical manifestations. Like the quinine-*plus*-plasmoquine treatment, this method has, however, the advantage of decreasing and devitalizing the gametocytes, especially those of *P. falciparum.* Moreover, from the clinical point of view, it diminishes substantially the number of relapses, both in *malignant tertian* and, more especially, in *benign tertian* and *quartan.*

II. PRACTICAL SUGGESTIONS FOR TREATMENT AND PROPHYLAXIS

Without presuming to lay down hard and fast rules, and while avoiding any interference with either the personal freedom of the doctor, who has to take the responsibility for the treatment of each case, or the initiative of the malariologists, who have to judge the different circumstances of the local epidemiology in the field, the Commission believes that it is in a position to give certain indications. Account should, however, first be taken of the following points, on which reservations have been made in the previous pages, with respect, not only to the individual treatment of patients, but generally also to the application of any therapeutic procedure: (1) the varying reactions of the different parasite strains of the same species and of patients to the drugs; (2) the special indications applicable to the parenteral administration of drugs; (3) the drawbacks of the synthetic products (yellow coloration of the skin by atebrin, toxicity of plasmoquine).

(*a*) Individual Treatment of Patients

It is always desirable that the doctor should be in a position to diagnose malaria and to determine the species of parasite concerned by a *microscopical examination of the blood.*

In ordinary cases of *P. vivax* (benign tertian) infections, it is almost immaterial whether quinine or atebrin is employed for treatment of the attack. Plasmoquine, associated with quinine or atebrin, or administered after these drugs, has no appreciably useful effect on the attacks, but seems to reduce the frequency of subsequent relapses.

The association of plasmoquine with quinine, or its administration after atebrin, is useful in *P. falciparum* infections, on account of its action on gametocytes and relapses.

(*b*) Treatment in the Field

Atebrin, when used for collective treatment in daily doses of 0·30 gm. (for adults) for 5 to 7 days, acts in the same way as quinine in daily doses of 1 gm. to 1·30 gm. for 5 to 7 days or more. There is no reason, save financial considerations, why either quinine or atebrin should be preferred. The manner in which collective treatment is conducted will depend upon the intensity of the endemicity, which is itself the resultant of a series of factors : the incidence of malignant tertian infection (*P. falciparum*), the virulence of the strains, the sensitiveness of the strains to the various drugs, the susceptibility of various population groups, etc.

Collective treatment with quinine or atebrin may usefully be accompanied or followed by plasmoquine treatment, in order to diminish the number of gametocytes and the risk of relapses.

The choice of the *basic drug* for collective treatment should be left to the public administrations or malariological organizations which undertake or control such treatment, and will be guided by local and economic considerations as well as by the preferences of the medical profession and of the population. It should, however, be remembered that the choice of drug, as well as dosage and duration of therapeutic administration, should, so far as possible, be directed towards the achievement of the real aim of mass treatment. This aim is : (1) to secure the largest number of complete cures in cases of malaria (*intensive treatment of patients*) ; (2) to reduce to a minimum the risk of anopheline infection, either by direct action upon the gametocytes, or by indirect action on the parasites generally, thereby effecting an eventual reduction in the number of carriers of sexual forms (*gametocyte therapy*). It would therefore be wise not to rely on plasmoquine alone for this second purpose. These considerations also apply to the choice of the basic drug and the manner of its administration to the community, either directly associated with, or followed by, plasmoquine.

There are, however, large malarial areas, especially in the tropics, where such mass treatment is impossible of practical application for various reasons, often financial. Under such conditions, it is desirable to provide *adequate and easily available treatment for the clinical manifestations* of the disease, so that the morbidity, the mortality and the physical incapacitation of the afflicted individuals are diminished. Such treatment has very often to be placed in the hands of

laymen for distribution, and little or no direct medical supervision is possible. In these circumstances, the cinchona alkaloids appear to be the more suitable drugs.

(*c*) Mass Drug Prophylaxis

Mass drug prophylaxis has a twofold purpose : (1) to protect the population undergoing prophylactic treatment from the clinical manifestations of endemic malaria, in order that its working capacity and comparative standard of health may be safeguarded without injury to its premunization, even in areas in which it is exposed to repeated reinfection ; (2) to reduce, in due course, the sources from which the local mosquitoes may be infected.

No prophylactic method, unless applied to disciplined communities under stringent supervision, is capable as yet of attaining these two objects. Special stress should be laid upon the desirability—and, at the same time, the difficulty—of promoting the immunization process in the population, which depends precisely upon the degree of tolerance to infection and upon repeated reinfection. At the same time one wishes to avoid the risks attendant upon the presence and persistence of such latent infections in the community.

Experience has shown, at all events, that very useful results can be obtained with *daily doses of quinine* (0·40 gm.) administered during the whole of the malaria transmission season, and even for a few weeks longer. This is also true of *bi-weekly doses of atebrin* (0·20 gm. = 0·40 gm. per week) administered in certain conditions. With the latter method (bi-weekly atebrin), which has given encouraging results, further trials would be desirable. The daily dose of 0·05 gm. of atebrin recommended for prophylactic purposes has proved inadequate. The harmlessness of quinine makes it a suitable drug for administration by subordinate personnel without constant medical supervision, whereas such supervision is essential in the case of atebrin.

Plasmoquine should not be distributed for prophylactic treatment otherwise than under direct medical control. Its use in mass prophylaxis would be justifiable only if it were administered to a disciplined population, which should be kept under constant medical care. It is useful more especially for the purpose of ultimately reducing the number of gametocyte carriers and arresting the transmission of infection to the *Anopheles*. Plasmoquine is certainly the gametocidal agent *par excellence*, especially where *P. falciparum* is concerned. But both quinine and atebrin also exercise in the latter case a gametocidal action (mainly indirect) by destroying the sexual forms in process of development.

The real efficiency of such methods in the field is, moreover, largely dependent upon a highly important epidemiological factor, namely, *the children*. The proportion of gametocyte carriers is much higher among children than among adults, given the same environmental conditions (village, house, family) in respect of endemicity and anophelism. Children, on the other hand, are more difficult to subject to regular treatment, and, finally, as already pointed out,

the *doses* and *form of administration* of atebrin and plasmoquine to children cannot yet be regarded as finally settled.

(*d*) Drug Eradication

Experience has so far shown that the eradication of malaria from a locality by the curative and prophylactic treatment, with the drugs at present available, is practically impossible. To begin with, it is impossible to reach, in sufficient time, all the inhabitants of an area, or even of a small village. Moreover, while curative and prophylactic treatment *may greatly diminish the morbidity, yet it cannot suppress the parasites in all the carriers.*

In this report, the Commission has not considered the question of expenditure entailed by treatment and prophylaxis campaigns, which depends upon the price of drugs and the cost of staff. It would, however, emphasize the great importance of this problem—which will probably be studied in detail by the Malaria Commission in the near future—in connection with the choice of drugs to be used for curative or prophylactic mass treatment.

Among those drugs, quinine still ranks first in current practice, by reason of its clinical effectiveness and almost complete absence of toxicity, coupled with the widespread knowledge of its use and dosage. As regards the synthetic products, which have only been used in therapeutics for 10 years, the Commission hopes that it has discharged the duty which devolved upon it by giving in this fourth general report an account of the present state of our knowledge regarding the possible use of *atebrin* and *plasmoquine* in the treatment and prophylaxis of malaria. In certain circumstances, as has been shown above, these drugs—representing a notable scientific advance—possess a very special value.

REFERENCE

League of Nations (1937). The treatment of malaria: fourth general report of the Malaria Commission. *Bull. Health Org.*, **6**, 895.

THE MOUTH-PARTS OF THE FEMALE IN *SIMULIUM DAMNOSUM* THEOBALD, WITH SPECIAL REFERENCE TO THE TRANSMISSION OF *ONCHOCERCA VOLVULUS* LEUCKART

BY

E. G. GIBBINS

(*Received for publication January 10th*, 1938)

CONTENTS

	PAGE
INTRODUCTION	9
THE MANDIBLES...	11
THE MAXILLAE	14
THE LABRUM-EPIPHARYNX AND HYPOPHARYNX	14
THE LABIUM	19
SUMMARY...	19
REFERENCES	20

INTRODUCTION

A little more than a decade ago Blacklock (1926*a*) incriminated *Simulium damnosum* Theobald in the transmission of *Onchocerca volvulus* Leuckart. This important discovery presented certain unusual features, since most blood-sucking insects derive the parasite from the blood-stream, whereas the microfilariae of *Onchocerca volvulus* are found beneath the surface of the skin. Blacklock's study of the mouth-parts, together with certain observations made by him on the insects' behaviour when in the act of biting, did much to elucidate the peculiar circumstances in which *Simulium* become infected. In order, however, more fully to understand this phenomenon, it appeared desirable that a detailed study of the mouth-parts should be undertaken.

Among the insects which torment man there is perhaps none which inflicts so cruel a bite as *Simulium damnosum*. The severity of the bite has been remarked upon by many travellers in Africa. Speke (1863), when describing his epic discovery of the source of the River Nile in Uganda, states that ' A small black fly, with thick shoulders and bullet-head infests the place and torments the naked arms and legs of the people with its sharp stings to an extent that must render life miserable to them ' ; and Balfour (1906) writes that the insect bites fiercely and is a veritable terror in certain parts of the Sudan.

The mouth-parts of *Simulium* have been studied by Cragg (1913), Emery (1913), Cameron (1922), Blacklock (1926*a*), Jobling (1928), the present writer (1933), Dorogostajskij *et al.* (1935), and Smart (1935) ; but, apart from the pioneer work of Cragg and the comparative study by Jobling, the various workers confined their investigations to a somewhat brief examination of the parts.

Throughout the present study, the nomenclature adopted is that of Jobling (1928), who went carefully into the question of homologies and terminology in

his exhaustive study of the mouth-parts of *Culicoides*. For a complete account of the various structures the reader is invited to consult this admirable work. The main purpose of the present inquiry is to attempt to demonstrate the manner in which the mouth-parts are used during the process of biting and blood-sucking. There is little hope of solving the problem by direct evidence, as the parts are hidden during the operation of biting.

The so-called act of biting appears to be performed in two stages. First, the initial incision is made by the mandibles, which function in the manner of a pair of scissors ; the maxillae are then inserted and the puncture is enlarged sufficiently to allow the food channel to reach blood-level.

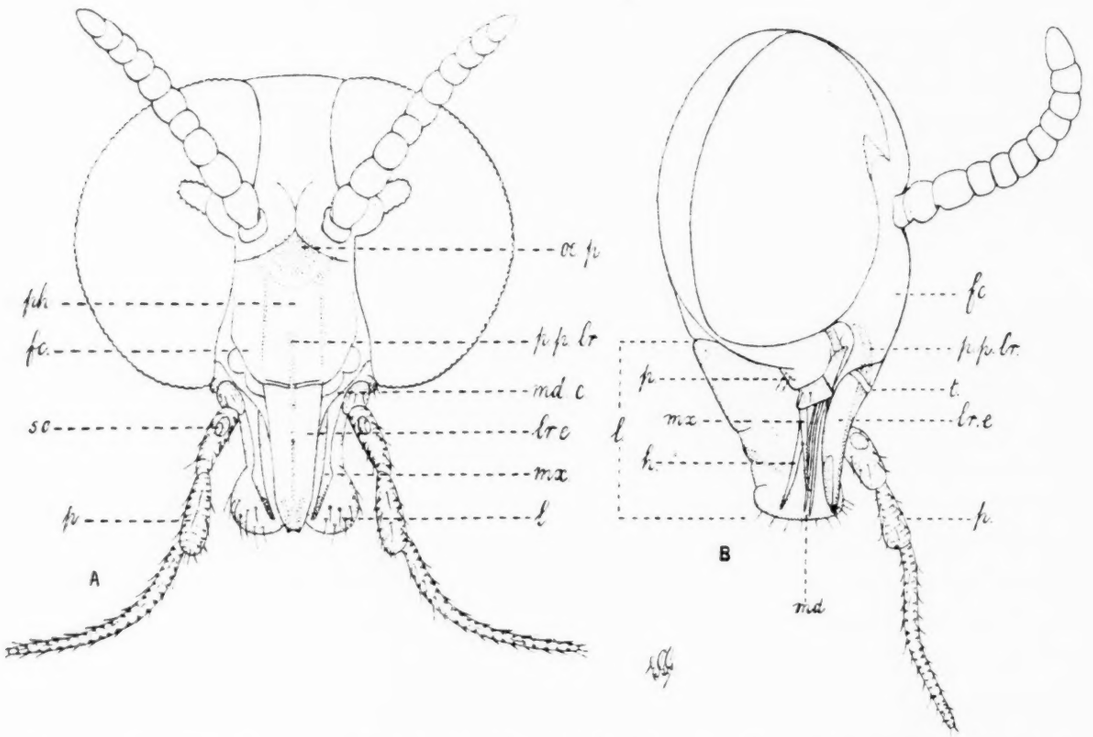


FIG. 1. *Simulium damnosum* Theobald. Head of female : A.—Dorsal view ; B.—Lateral view : labium and segments 3–5 of palp from one side removed, to show normal position of parts.

fc.—fronto-clypeus.
h.—hypopharynx.
l.—labium.
lr.e.—labrum-epipharynx.
md.—mandibles.
md.c.—mandibular condyle.
mx.—maxilla.
oe.p.—oesophageal pump.
p.—palp.
ph.—pharynx.
p.p.lr.—posterior process of labrum.
s.o.—sense organ.
t.—torma.

In *Simulium* the mouth-parts are relatively short. They comprise the same parts as are present in other blood-sucking Nematocera, and most closely resemble those of the genus *Culicoides*. They consist of the paired mandibles and maxillae, the labrum-epipharynx, the hypopharynx and labium. A general idea of the position of the respective mouth-parts in relation to the rest of the head may be obtained from fig. 1. The head has been drawn from a dorsal

aspect in fig. 1, A, and fig. 1, B, depicts the head in lateral view ; in the latter figure one side of the labium and segments 3–5 of one palp have been dissected away, leaving a clear view of the biting parts. Only one maxilla is shown, but both mandibles are figured in their normal position.

THE MANDIBLES

The mandibles of *Simulium* are similar to those of *Culicoides* and take up a position in the middle line, right over left, between the labrum-epipharynx and the hypopharynx (fig. 1, B), as shown in cross-section by Jobling (1928) in the case of *Simulium ornatum*. They articulate with the mandibular condyle, a process attached on either side of the fronto-clypeus. The mandible (fig. 2, B) is thin and blade-like, with the anterior end finely grooved and slightly curved inwardly. At the distal extremity of each mandible is an oblique row of flat teeth, which are directed inwards and resemble the edge of a saw. In *Simulium damnosum* and *Simulium neavei*, the two most important human blood-suckers, the teeth are confined almost entirely to the inner apical edge, but in many species they continue a short distance round the outer edge, as shown in fig. 3. Some small variation in the general shape of the structure has also been noted among other species.

Running from the base of the mandible along, but free of, the outer edge is a narrow tendon—the abductor of the mandible—which terminates slightly anterior to a curious process lying within but slightly towards the outer side, termed by Jobling the ' depression.' Though forming a depression on its upper (dorsal) surface, it gives rise to a knob-shaped process on the under side (fig. 2, C), which is actually the axis on which the organ articulates. Jobling made a cross-section at this juncture in the case of *Culicoides*, and showed that the two blades are interlocked. In *Simulium* the blades are invariably found separated when the head is examined after being dechitinized, and it would appear that the fly can, at will, cause the blades to part. Whether this is a normal procedure after biting and the blades are drawn to each side in order to free the labrum-epipharynx and hypopharynx to form the food channel, as said by Adler and Theodor (1926) to be the case in *Phlebotomus*, is not quite certain, as Jobling (1928) is of the opinion that they remain between the labrum-epipharynx and hypopharynx in *Culicoides* and form the ventral wall of the food channel.

How well the mandibles can function as a pair of scissors will be seen in fig. 4, which has been drawn with the two blades superimposed one above the other. The saw-like distal border makes a very effective cutting edge, and the two blades working together would undoubtedly make the required snip. There is every reason to believe that this is, indeed, the manner in which the initial puncture is made ; the mandibles do not pierce the skin, but make an incision with a snip-like action.

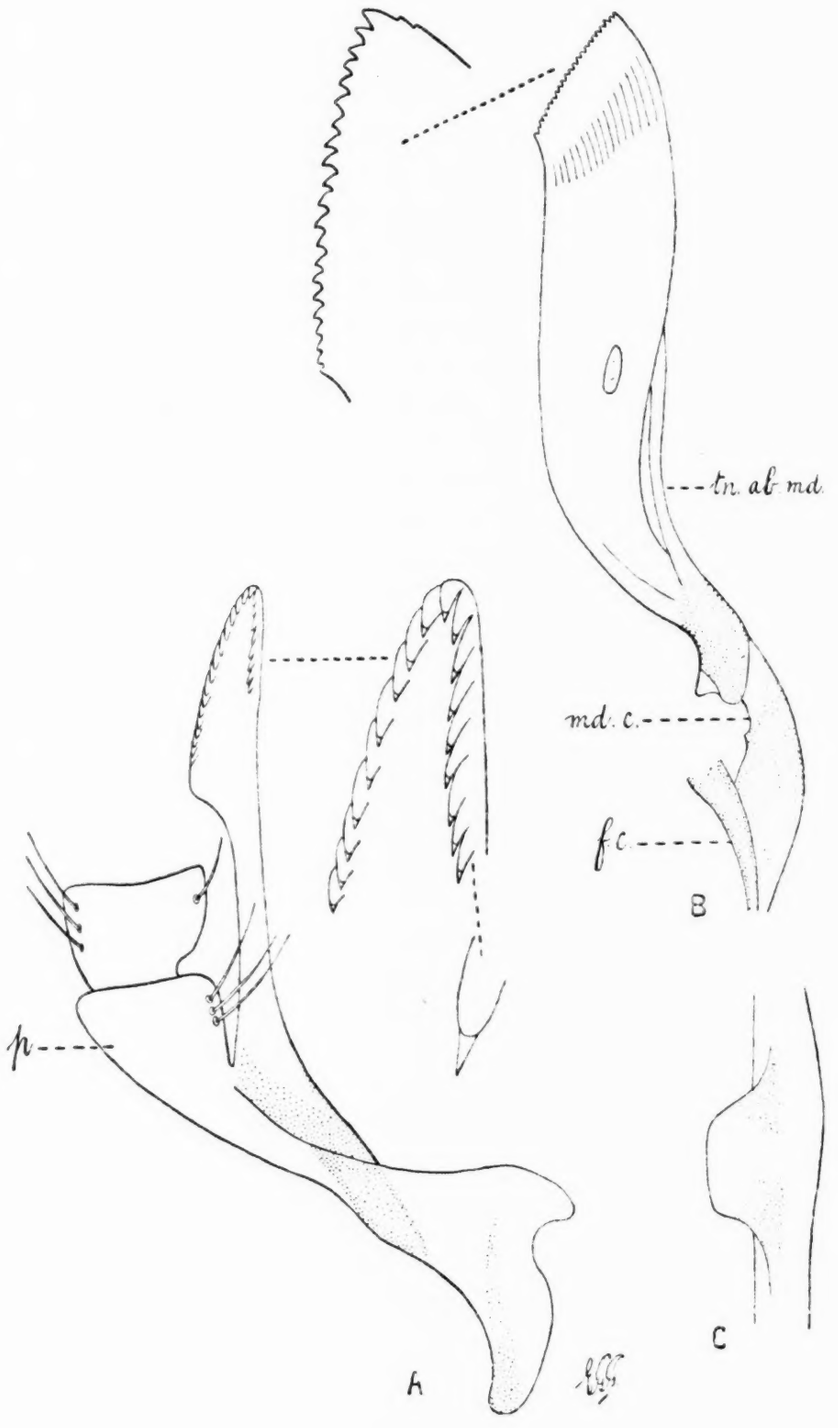


FIG. 2. A.—Maxilla; B.—Mandible; C.—Lateral view of median knob-shaped process of mandible. (Magnification of A and B as in fig. 5; of C as in fig. 8, C.)

fc.—fronto-clypeus.
md.c.—mandibular condyle.
p.—palp.
tn.ab.md.—tendon abductor of mandible.

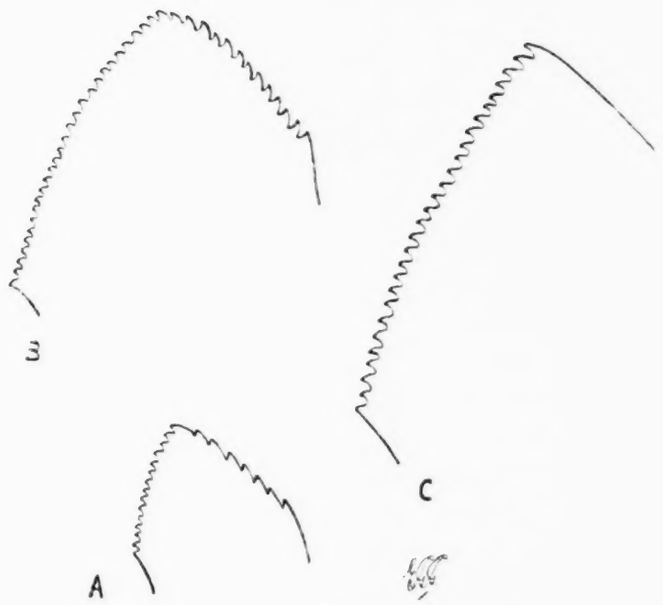


FIG. 3. Distal end of mandible. A.—*Simulium griseicolle* Becker ; B.—*Simulium dentulosum* Roubaud ; C.—*Simulium neavei* Roubaud. (Magnification as in fig. 8, C.)

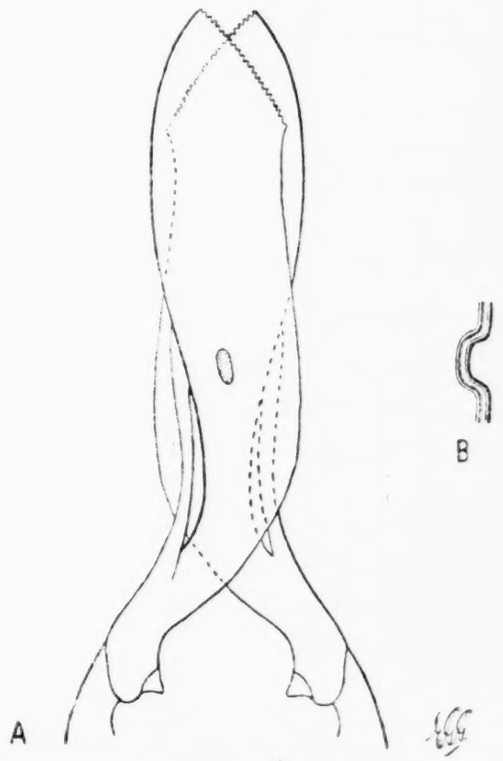


FIG. 4. A.—Mandibles, drawn to show their scissor-like arrangement ; B.—Diagrammatic representation of the knob-shaped central axis in lateral view. (Magnification of A as in fig. 5.)

THE MAXILLAE

Having progressed so far, it is apparent that an incision of such small dimensions would require to be considerably enlarged before it would be in a position to allow the labrum-epipharynx and hypopharynx to reach blood-level. It is then assumed that the maxillae, whose distal extremities lie just above the apex of the mandibles, come into play and tear the tissue asunder.

Cragg (1913) and Jobling (1928) have dealt with the various parts comprising the maxillae. For the purpose of the present study consideration of that part known as the galea will suffice.

The formidable armature of the maxilla is clearly seen in fig. 2, A. The strong recurved teeth, each terminating in a spine, are set in the form of a V along the outer edge ; they vary in number in different species. In *damnosum* there are less on one side than on the other, but in some species they are of equal number on both sides. The under surface is always entirely free from projections, and the anterior end is slightly curved inwardly (fig. 1, A).

It will be clear that, while this structure makes a very efficient tearing apparatus, it is not used for piercing or cutting. That its use lies more in enlarging the initial incision made by the mandibles, by tearing at the edge rather than in scratching at the surface of the skin, is suggested by the recurved teeth and by the fact that they operate in a more or less vertical position. In the case of *Culicoides* Jobling came to the conclusion, after a study of the muscles of the maxillae, that their main movement consists of protraction and retraction.

Most probably it is during this process of enlarging the initial puncture in order to accommodate the food channel that the microfilariae of *Onchocerca volvulus* are liberated from the skin. That great destruction of tissue does take place is borne out by Blacklock (1926*a*), who made dissections of *Simulium* killed in the act of biting but prior to the stage at which blood is imbibed, and who found cellular elements within the gut.

THE LABRUM-EPIPHARYNX AND HYPOPHARYNX

When the blood-sucking parts of *Simulium damnosum* are carefully removed from the head, the mandibles, which normally interpose between the labrum-epipharynx and hypopharynx, stay behind. The parts then appear from a lateral view, as shown in fig. 5.

The labrum-epipharynx may be likened to a narrow upright test-tube, whose wall is of delicate membrane strengthened by three long strips of chitin, i.e., the labrum, and flattened and slightly grooved on the inner (ventral) side to form the epipharynx.

The labrum is composed of two parts : the lateral part, which is a paired structure with the epipharynx lying between, and the median part, which forms the dorsal stay of the structure. The median part of the labrum (fig. 6, A) is a long chitinized rod-like process expanded distally and armed with two groups of three heavily chitinized curved teeth ; these are directed forwards and the

tips are bent up to form a hook. Cragg (1913) was of the impression that the teeth were used to fix the organ in position once the labrum-epipharynx had eached a suitable depth, a view with which the present writer entirely agrees. The median part of the labrum enters the head capsule in its proximal third,

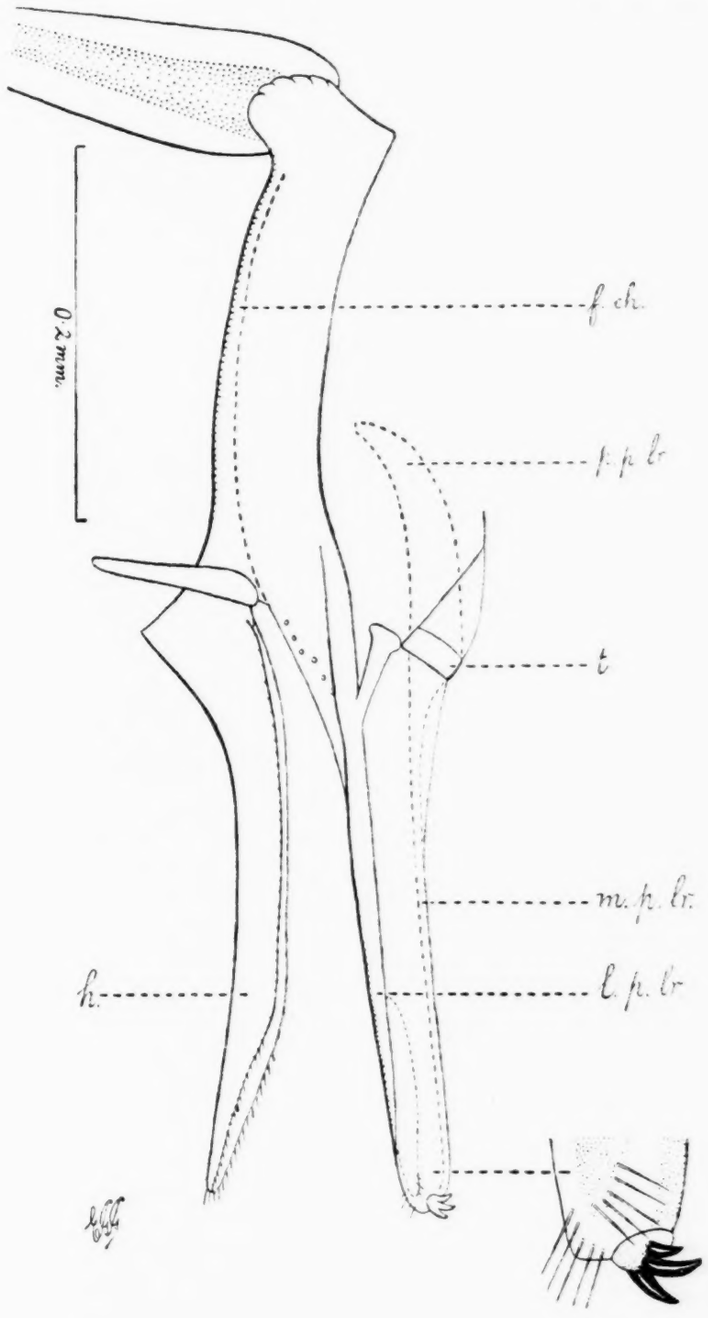


FIG. 5. Labrum-epipharynx and hypopharynx in lateral view.
f.ch.—food channel.
h.—hypopharynx.
l.p.lr.—lateral part of labrum.
m.p.lr.—median part of labrum.
p.p.lr.—posterior process of median part of labrum.
t.—torma.

where it is free, and assumes the shape of a small india-rubber teat when seen in dorsal view. Fig. 6, B, shows the inner ventral surface of the labrum-epipharynx. The lateral parts of the labrum are flat and bar-like, broadening gradually anteriorly where they are fused with a large fish-head-like process bearing a submedian row of three spicules on either side. The distal extremity (fig. 6, C) is gently rounded and armed with rather broad unchitinized spines, with two groups, each with three minute spicules on either side at the tip.

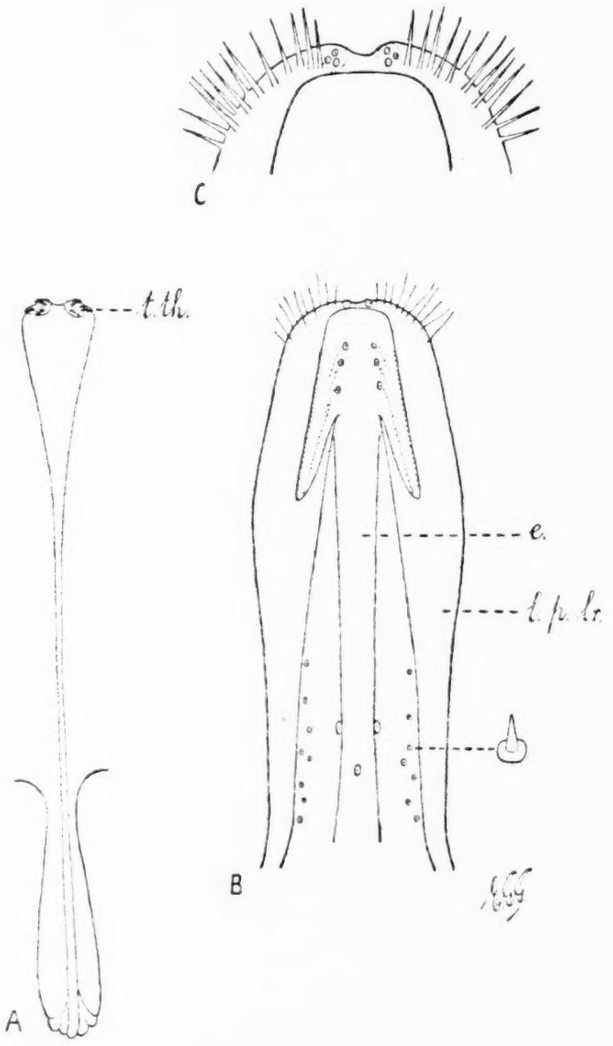


FIG. 6. Labrum-epipharynx. A.—Median part of labrum in dorsal view: *t.th.*—terminal teeth; B.—Ventral view of lateral parts of labrum (*l.p.lr.*) and epipharynx (*e*); C.—Enlarged view of distal extremity of B. (Magnification of A and B as in fig. 5; of C as in fig. 8, C.)

The epipharynx, which is narrow and well-chitinized, is attached to the lateral parts of the labrum by a transparent membrane bearing an oblique irregular row of fine non-pigmented spicules; these are difficult to discern, and when seen in optical section appear as clear spaces with a dark central spot.

Shaped rather like a garden trowel, the hypopharynx (fig. 7) is a rigid structure which bears the salivary duct in the median line of its inner surface. On either side of the duct, continuing obliquely to the tip, is a slightly raised strip of chitin which, near its anterior end, bears a single row of closely placed, long, fine hairs which are directed laterally. Around the distal edge is a double row of downwardly directed spines; those on the inner surface are strong,

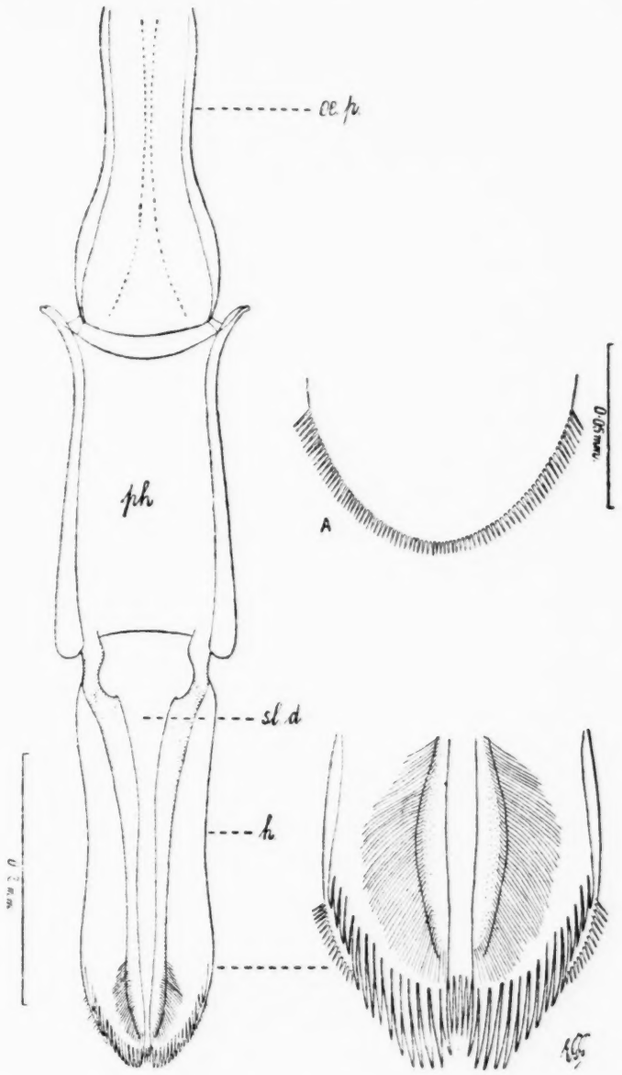


FIG. 7. Hypopharynx in dorsal view. A.—Ventral surface of distal extremity of hypopharynx.

h.—hypopharynx.
oe.p.—oesophageal pump.
ph.—pharynx.
sl.d.—salivary duct.

heavily chitinized and somewhat lanceolate in shape, and except for a few in the centre, which are distinctly shorter than their immediate neighbours, they are long and gradually diminish in length as they continue laterally, terminating a short distance inside the outer margin. Those on the ventral surface (fig. 7, A) keep entirely to the edge and are short and unchitinized.

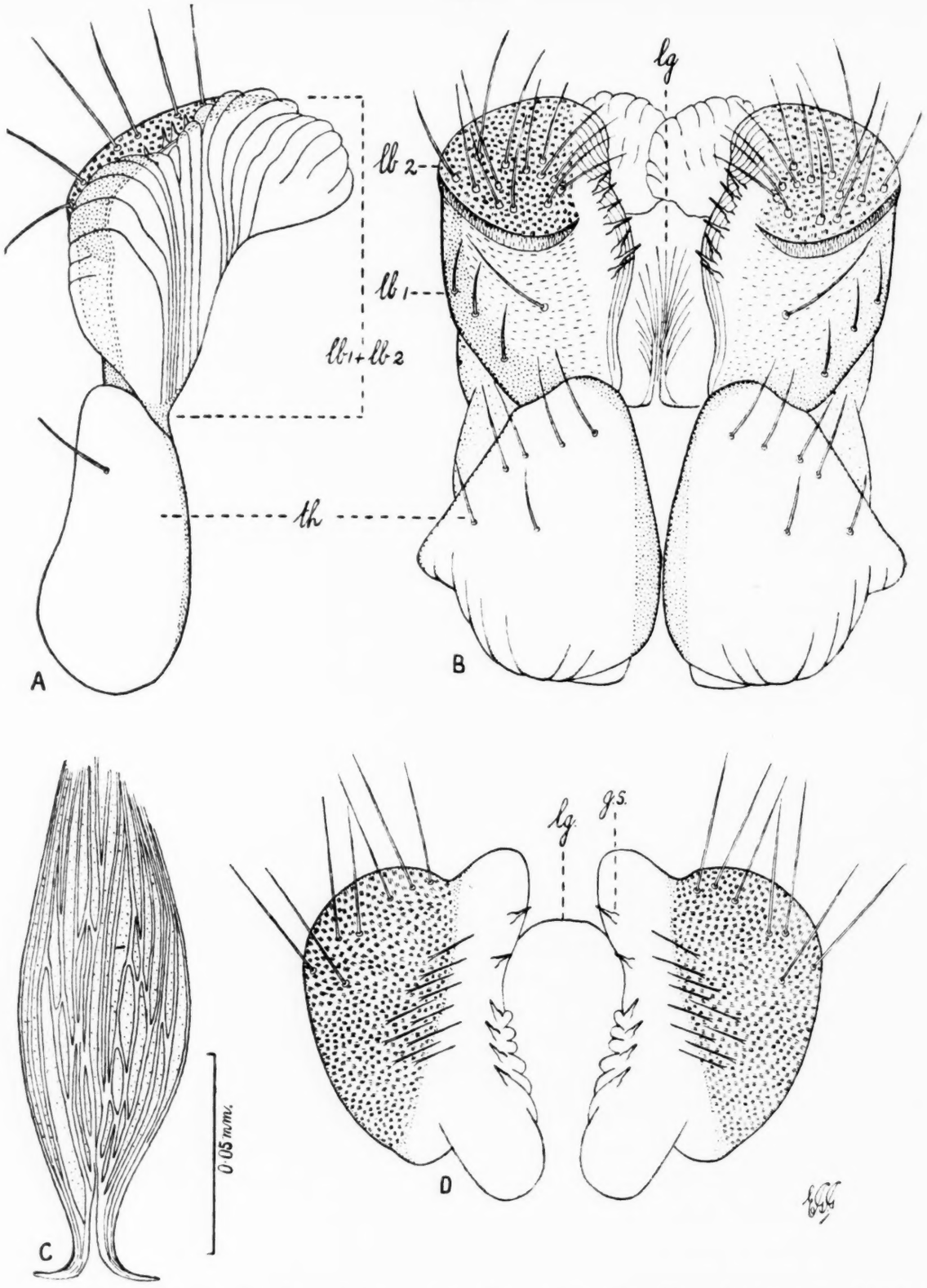


FIG. 8. Labium. A.—Lateral inner view of one side; B.—Ventral view; C.—Ligula; D.—View from above, looking down on the distal extremity: note the intervening space, which is normally occupied by the biting and blood-sucking parts. (Magnification of A, B and D as in fig. 5.)

g.s.—gustatory sensilla.
lg.—ligula.
lb.—labellum.
th.—theca.

Cragg (1913) suggested that the spines at the apex of the labrum-epipharynx and hypopharynx form an efficient means of preventing large particles from entering the food channel. While they may act as a sieve, they also form an effective trap for wandering *Onchocerca*, which would eventually find their way into the food channel.

THE LABIUM

The labium of *Simulium damnosum* is a large fleshy process which is attached to the head capsule near the base. In their normal position the biting parts lie between the right and left labella, in the space clearly seen in fig. 8, D, which depicts the labium end on, looking down from above, with the distal end of the second labella on either side. The first and second labella are fused and, although strongly chitinized outwardly, with the two segments clearly demarcated the inner surface (fig. 8, A) is comprised of a very delicate concertina-like membrane, with the rays running vertically in a continuous line from the base of the first to the tip of the second labellum ; anteriorly the membrane is produced into a small dorsal projection. Blacklock (1926*b*) records the microfilariae of *Onchocerca volvulus* emerging from one or other side of the median line at the anterior margin of the labella, and it is undoubtedly the rupture of this delicate membrane which provides them with an easy exit. Between the labella, forming a ventral wall, is the ligula, whose peculiar branched vein-like structure can be seen in fig. 8, C.

SUMMARY

1. The mandibles, maxillae, labrum-epipharynx, hypopharynx and labium of the female in *Simulium damnosum* Theobald are described and figured in detail.

2. It is suggested that the so-called ' act of biting ' is performed as follows : first, the mandibles, functioning as a pair of scissors, snip the skin ; the maxillae are then thrust within the incision, which is enlarged by a tearing process until it is of sufficient size to allow the labrum-epipharynx and the hypopharynx to attain blood-level.

3. The microfilariae of *Onchocerca volvulus* are liberated from the skin by the maxillae during the process of enlarging the initial incision.

4. The numerous spines at the tip of the labrum-epipharynx and hypopharynx form an effective trap for wandering microfilariae.

5. A detailed study of the labium has shown the extremely delicate nature of the inner surface of the labella, through which the *Onchocerca* find an easy exit.

Acknowledgement.—It is a pleasure to record my indebtedness to Professor D. B. Blacklock, who very kindly read through the manuscript of this paper.

REFERENCES

ADLER, S., and THEODOR, O. (1926). The mouth parts, alimentary tract, and salivary apparatus of the female in *Phlebotomus papatasii*. *Ann. Trop. Med. & Parasitol.*, **20**, 109.

BALFOUR, A. (1906). Second report of the Wellcome Research Laboratories at the Gordon Memorial College, Khartoum. p. 34. Khartoum.

BLACKLOCK, D. B. (1926*a*). The development of *Onchocerca volvulus* in *Simulium damnosum*. *Ann. Trop. Med. & Parasitol.*, **20**, 1.

——— (1926*b*). The further development of *Onchocerca volvulus* Leuckart in *Simulium damnosum* Theob. *Ibid.*, **20**, 203.

CAMERON, A. E. (1922). The morphology and biology of a Canadian cattle-infesting black fly, *Simulium simile* Mall. (Diptera, Simuliidae). *Bull. Dept. Agric., Canada, new ser.*, no. 5.

CRAGG, F. W. (1913). Studies on the mouth parts and sucking apparatus of the blood-sucking Diptera. II : Some observations on the morphology and mechanism of the parts in the Orthorrapha. *Sci. Mem. Med. Sanit. Dept. India*, no. 58.

DOROGOSTAJSKIJ, V., RUBZOV, I., and VLASENKO, N. (1935). Notes on taxonomy, biology and geographical distribution of black flies in East Siberia. [In Russian ; English summary.] *Mag. Parasitol., Leningrad*, **5**, 107.

EMERY, W. T. (1913). Morphology and biology of *Simulium vittatum* and its distribution in Kansas. *Kansas Univ. Sci. Bull.*, **8**, 323.

GIBBINS, E. G. (1933). Studies on Ethiopian Simuliidae : *Simulium damnosum*, Theo. *Trans. Roy. Ent. Soc. Lond.*, **81**, 37.

JOBLING, B. (1928). The structure of the head and mouth parts in *Culicoides pulicaris*, L. (Diptera, Nematocera). *Bull. Ent. Res.*, **18**, 211.

SMART, J. (1935). The internal anatomy of the black-fly, *Simulium ornatum* Mg. *Ann. Trop. Med. & Parasitol.*, **29**, 161.

SPEKE, J. H. (1863). A journal of the discovery of the source of the Nile. p. 465. Edin.

NOTES ON ETHIOPIAN SIMULIIDAE.—II

BY

E. G. GIBBINS

(*Received for publication January 10th*, 1938)

The present contribution, which is the second in this series of notes on the Ethiopian Simuliidae, is concerned mainly with the study of *Simulium* in the national collection at the British Museum and those in the possession of the South African Museum at Cape Town. The writer is greatly indebted to Dr. F. W. Edwards, for his kindness in facilitating the study of the London collection, and to Dr. K. H. Barnard and Dr. A. J. Hesse, for the loan of adults from South Africa. Dr. Barnard, who has taken a great interest in the Simuliid fauna of South Africa, also placed at the writer's disposal an extensive collection of the early stages, together with many valuable records. Dr. B. De Meillon, of the South African Institute for Medical Research, and Monsieur Séguy, of the Muséum National d'Histoire Naturelle, Paris, facilitated the study by their kind co-operation in sending *Simulium*. The inclusion of new records from the Gold Coast is due to the kindness of Sir Guy A. K. Marshall, of the Imperial Institute of Entomology, who sent *Simulium* from that country for determination. My thanks are also due to Dr. S. A. Neave, of the same Institute, and to Miss G. Phillips, Librarian to the Liverpool School of Tropical Medicine, for kind assistance with the literature.

The paper deals with five new species and the synonymy and distribution of the *Simulium* in the collections studied. In the case of the new species, only tentative diagnoses are given, as the writer is engaged on a revision of the Ethiopian Simuliidae. In view of the delay which has occurred in the publication of a paper dealing with Simuliidae from the Ruwenzori Mountains of Uganda, diagnoses of the new species described therein are included in the present communication. The new species dealt with include *S. barnardi* and *S. turneri* from South Africa, and *S. kauntzeum*, *S. taylori* and *S. bisnovem* from Uganda. The following species have been found to be synonymous : *S. gilvipes* Pomeroy and *S. ruwenzoriensis* Gibbins with *Simulium dentulosum* Roubaud ; *S. ugandae* Gibbins, *S. capensis* Enderlein and *S. pseudomedusaeformis* De Meillon with *Simulium medusaeformis* Pomeroy ; *S. vorax* Pomeroy with *Simulium neavei* Roubaud ; *S. caffrarica* Enderlein with *Simulium nigritarsis* Coquillett ; and *S. tisiphone* De Meillon with *Simulium elgonensis* Gibbins. It has become necessary to use the name *S. impukane* De Meillon, hitherto regarded as a synonym for the African *violaceum* of Pomeroy, as Enderlein's European species of that name has priority. Similarly, *S. cavum* Gibbins is adopted for the African *obscurum*, as the writer had overlooked the fact that Enderlein had already used this name to signify a variety of *S. tristrigata*. In addition to the

usual data, the donors of certain specimens in the collection of the British Museum included valuable notes on the circumstances of capture. These hitherto unpublished observations are recorded below and greatly enhance our knowledge of the blood-sucking habits of *S. adersi*, *S. alcocki*, *S. bovis*, *S. griseicolle*, *S. neavei* and *S. neireti*.

Simulium barnardi sp. nov.

The early stages of the fly were obtained by Dr. K. H. Barnard, in whose honour the species is named. Amongst the material were mature pupae, from which it was possible to dissect the adult of both sexes, and, though the flies were of little use for the purpose of an external description, the terminalia were fully developed and showed the unique character of the insect.

In the female the species is readily distinguished from all other Ethiopian *Simulium* by the very heavily chitinized eighth sternite, which is produced anteriorly on either side of the usual median depression into a broad flat process; while in the male the presence of a row of six teeth near the apex of the clasper renders this sex easily identifiable.

The respiratory organ of the pupa is composed of 15 uniformly long and narrow semi-translucent filaments with a finely striated outer wall; 12 of the filaments are arranged in pairs.

HOLOTYPE male, on slide, dissected from pupa. CAPE. Clanwilliam, Cedar Mountains, October, 1934, K. H. Barnard.

ALLOTYPE female, on slide, dissected from pupa, same data.

LARVAE and PUPAE. CAPE. Tulbagh, Great Winterhoek Mountains, November, 1932; Clanwilliam, Cedar Mountains, October, 1934, K. H. Barnard.

Simulium turneri sp. nov.

Two females were collected by Mr. R. E. Turner from the Cape Province of South Africa in September, 1928. The species is named in honour of Mr. Turner.

Simulium turneri is a dark insect about 3 mm. in length; its claws are supplied with a basal projection, and the membrane behind the mesothoracic spiracle is without hairs. The characteristic terminalia and the presence of very long, narrow, pale-yellow, hair-like scales which densely cover the abdomen and legs distinguishes it immediately from all other Ethiopian Simuliids.

HOLOTYPE female. CAPE. Worcester, September, 1928, R. E. Turner.
PARATYPE female, same data.

Simulium kauntzeum sp. nov.

A large species, closely resembling *S. nili* Gibbins in having a patch of hairs on the membrane behind the mesothoracic spiracle and a pronounced basal projection of the claws. Among other characters, the female fly may be best distinguished on the general colour of the abdomen, which, though shining in both cases, is light brown in *kauntzeum* and black in *nili*.

The male resembles both *S. dentulosum* Roubaud and *S. nili* Gibbins, but may be easily distinguished from these two species by the absence of the slate-grey lateral patch on the fifth and sixth abdominal segments and by the extremely large first tarsal segment of the hind leg.

The respiratory organ of the pupa is very characteristic. It is composed of nine long stout filaments with deeply pigmented tips; eight of the filaments are paired and in addition a short stumpy spine-like process projects near the base. The outer wall is similar in sculpture to *S. bisnovem* sp. nov.

HOLOTYPE male, bred from isolated pupa. UGANDA. Mount Ruwenzori, Namwamba Valley, Kiriruma, 10,200 ft., December 25th, 1934, E. G. Gibbins.

ALLOTYPE female, same data.

PARATYPES 8 males, bred, same data as types. Four males, 1 female, caught, Kiriruma, 10,200 ft., F. W. Edwards.

Simulium bisnovem sp. nov.

Though no description of the species has yet appeared, this insect has previously been referred to as *S. edwardsi* (Gibbins, 1937*a*). Dr. De Meillon, however, has called my attention to the fact that the name *edwardsi* is pre-occupied in *Simulium*, having been used by Enderlein.

The female is a large reddish-brown *Simulium* measuring about 4·5 mm. in length. There are no hairs on the membrane behind the mesothoracic spiracle, and the claws have a basal projection. From *S. debegene* De Meillon, to which it bears a resemblance, it may be distinguished by the presence of three dark-blue longitudinal stripes on the mesonotum.

In the male the characteristic heart-shaped anterior part of the phallosome readily separates it from all other species.

The respiratory organ of the pupa is composed of 18 stout paired filaments with deeply pigmented tips and a finely striated outer wall similar to *S. nili* Gibbins, from which it may be distinguished by the larger number of filaments (*S. nili* has 14).

HOLOTYPE male, bred from isolated pupa. UGANDA. Mount Ruwenzori, Namwamba Valley, Kyanjoke, 6,500 ft., December 26th, 1934, E. G. Gibbins.

ALLOTYPE female, same data.

PARATYPES male, bred from pupa. UGANDA. Foothills of Mount Ruwenzori, Bwamba Pass, November 17th, 1931, E. G. Gibbins. One male, caught, Mount Ruwenzori, Namwamba Valley, Kyanjoke, 6,500 ft., F. W. Edwards.

Simulium taylori sp. nov.

The species bears a very close resemblance to *S. cavum* Gibbins, but may be distinguished in the terminalia of the male and, externally, by the long dark-brown hairs of the lateral fringe of the first abdominal tergite ; these hairs are pale yellow in *cavum*.

Though bearing such a close resemblance to *cavum* in the adult, the respiratory organ of the pupa is very different. From a main, slightly chitinized, broad, basal appendage arise 15 slender semi-translucent filaments ; these arise singly, with the exception of 7, which originate from two short stems on the dorsal surface and support a pair and 5 filaments respectively, one of the latter group being considerably shorter than the rest.

HOLOTYPE male, bred from isolated pupa. UGANDA. Foothills of Mount Ruwenzori, Bwamba Pass, November 17th, 1931, E. G. Gibbins.

ALLOTYPE female, same data.

PARATYPES 5 males, 1 female, same data.

Simulium adersi Pomeroy

This insect, which was first recorded by Pomeroy (1922) as a variety of *S. hirsutum* on the character of the respiratory organ of the pupa, was raised to the status of a species by the present writer (1934), who described the male, female, pupa, cocoon and larva and gave further details of the male terminalia (1936*a*). The species, which is a human blood-sucker, was breeding in peculiar circumstances in Uganda, the early stages being found in the wash of the waves on the rocky shores of the small islands in Lake Victoria. New records showing its presence on other islands in the lake and as far afield as the Transvaal are reported below.

Apart from the more silvery appearance of the pubescence on the mesonotum, the South African specimens agree closely with those from Uganda. A new record of the blood-sucking habits of the fly is furnished by C. C. Gowdey, who observed that the insect bites only at sunrise and sunset.

UGANDA. 1910, A. D. Fraser ; Lake Victoria, Damba Island, March 26th, 1911, G. D. H. Carpenter ; Kome Isle, April 11th, 1914, C. C. Gowdey ; Nkosi Isle, South Sesse, May 25th–27th, 1928, G. D. H. Carpenter.

ZANZIBAR. August 22nd, 1917, A. W. J. Pomeroy.

TRANSVAAL. Pretoria, March 13th, 1919, G. A. H. Bedford.

Simulium africanum Gibbins

The original description of the male and female was given by the present writer (1934), who also dealt with the larva, pupa, cocoon and breeding-places.

As the fly has hitherto been known only to occur in Uganda, the undermentioned record, based on the respiratory organ of the pupa, is not without interest as showing its presence at the Cape.

CAPE. Mitchell's Pass, Ceres, October, 1934, H. G. Wood and C. W. Thorne.

Simulium alcocki Pomeroy

Pomeroy (1922) made his original description of the species from specimens collected in Nigeria. It is very closely related to *S. duodecimum* Gibbins in the adult, but the respiratory organ of the pupa differs considerably.

The first observation on the blood-sucking habits of this small fly is given by Mr. E. Hargreaves, who took engorged adults from a fowl at Njala in Sierra Leone on April 17th, 1927.

SIERRA LEONE. Kaiyima, December, 1922, Panguma, December, 1923, Freetown, November, 1925, River Njia, January, 1926, Punderu Road, D. B. Blacklock; Njala, December, 1926, and April 17th, 1927, E. Hargreaves.

NATAL. Van Reenen, Drakensberg, January 1st-22nd, 1927, R. E. Turner.

NYASALAND. Cholo, R. C. Wood; Zomba, 1913, H. S. Stannus.

NIGERIA. Ibadan, December 11th, 1920, A. W. J. Pomeroy.

Simulium arnoldi Gibbins

The female and early stages of this *Simulium* are unknown. The species was recently described by the present writer (1937*b*) from examples collected in Rhodesia.

NATAL. March to April, 1924, H. P. Thomasset.

Simulium blacklocki Edwards

Closely related to *S. palmeri* Pomeroy (1922), the species is easily identified on the peculiar character of the respiratory organ of the pupa (De Meillon, 1930).

SIERRA LEONE. 1926, D. B. Blacklock.

Simulium bovis De Meillon

A small but characteristic blood-sucking species of both medical and veterinary importance. The female was first described by De Meillon (1930), and the male has since been described by the same writer (1935), who also dealt with the respiratory organ of the pupa.

Dr. A. Yale Massey took the fly when biting in the Belgian Congo, Mr. R. E. Lloyd captured the same insect while biting in Northern Rhodesia, and Mr. R. C. Wood took numerous specimens on cattle in Nyasaland.

BELGIAN CONGO. Coquilhatville, October 15th, 1910, A. Y. Massey.

NYASALAND. Lake Nyasa, south-eastern shore, March, 1910, S. A. Neave; Ruo, 200 ft., December 12th, 1914, April 24th, 1916, December 11th, 1916, and January 9th, 1917, R. C. Wood.

NORTHERN RHODESIA. Serenje Boma, River Kaomba, 4,200 ft., April, 1920, R. E. Lloyd.

ZULULAND. Umblatusi, April 4th, 1923, G. A. H. Bedford.

Simulium cervicornutum Pomeroy

Pomeroy (1920) described the male, female and respiratory organ of the pupa from specimens obtained in the Cameroons. The species was redescribed in all its phases (with the exception of the egg) by the present writer (1936*a*) from Uganda specimens, and has since been reported from the Belgian Congo (Gibbins, 1936*b*) and South Africa (De Meillon, 1935).

CAMEROONS. Bangan, February 14th, 1916, A. W. J. Pomeroy.

Simulium cavum nom. nov.

In naming the species *Simulium obscurum*, the present writer (1937*a*) overlooked the fact that Enderlein had already used the name to signify a variety of *Simulium tristrigata*. It is suggested that the African *obscurum* should be known in future as *Simulium cavum* Gibbins.

Simulium damnosum Theobald

The undermentioned females, which were all caught, agree with those of *damnosum* from the type locality in Uganda (Gibbins, 1933, 1937*a*).

BELGIAN CONGO. Near Leopoldville, December 13th, 1903, Dutton, Todd and Christy; Katanga, River Lufuka, 1,925 m., October 23rd, 1925; Stanleyville, October, 1927, J. Schwetz.

CAMEROONS. Tinto, January 10th, 1916, A. W. J. Pomeroy; Victoria, December 22nd, 1920, L. H. Booth.

GOLD COAST. Ashanti, April 10th, 1913, J. J. Simpson; Prestea, January, 1925, W. F. Chevers.

KENYA. Juja, August 14th, 1918, A. W. J. Pomeroy.

LIBERIA. May 21st, 1909, A. Pearse.

NYASALAND. Cholo, 3,500 ft., Limbe, December 26th, 1916, R. C. Wood.

SIERRA LEONE. Robarri, November 9th, 1905, W. H. Grattan; Maybole River, Karina District, October, 1906, H. E. Arbuckle; Iamadu, June 27th, 1912, J. J. Simpson; Dia, December 22nd, 1924, E. Hargreaves.

SUDAN. Abu Hamed, 1905, Talbot; Mvolo, Rumbek District, Bahr-el-ghazel.

TRANSVAAL. Pretoria, March 11th, 1915, G. H. A. Bedford.

UGANDA. Christy; 1909, D. Bruce; Mpumu, July, 1910, A. D. Fraser.

Simulium dentulosum Roubaud

Formerly (Gibbins, 1934) it was shown that *S. ruwenzoriensis* Gibbins was distinct from *S. dentulosum* Roubaud. A recent study of a long series of bred specimens from different localities in Uganda does not uphold this view, and *S. ruwenzoriensis* must therefore become a synonym of *S. dentulosum* Roubaud.

It has been my privilege to examine Pomeroy's material of *S. gilvipes*, together with the type. There is no doubt that this insect is also synonymous with *S. dentulosum*.

CAMEROONS. Bangan, February 14th, 1916, A. W. J. Pomeroy.

CAPE. Cape Town, Platteklip Stream, June 10th, 1934, K. H. Barnard ; Mitchell's Pass, October, 1934, Wood and Thorne ; Meiring's Poort, Zwartberg Range (north of Oudtshoorn), January, 1935, K. H. Barnard.

KENYA. South-east slopes of Mount Kenya, 6,000–7,000 ft., February 3rd–12th, 1911, S. A. Neave.

UGANDA. Siroko River, west foot of Mount Elgon, 3,000 ft., August 12th–14th, 1911, S. A. Neave.

Simulium elgonensis Gibbins

A critical study of the male and pupa of *S. tisiphone* De Meillon (1936) shows that this insect is synonymous with *S. elgonensis* Gibbins.

Simulium griseicolle Becker

Simulium griseicolle is the only Ethiopian Simuliid without hairs on the base of the radius. It was first described by Becker (1903), and was dealt with by the present writer (1935), who redescribed the male and female and furnished an account of the larva and pupa. Hitherto the species had been reported only from the Sudan and Uganda, but the undermentioned data show that it also occurs in West Africa.

Though the insect is often troublesome to man, there is still no definite record of it sucking human blood. The following unpublished notes on the habits of the adult are made by various donors of specimens. In the Sudan Mr. H. W. Bedford found the insect flying round the ears and eyes of man in the early morning at Shambat, and also obtained specimens indoors upon the window-panes at Khartoum ; but he did not observe the fly to bite. There are, however, several records of this species engorged with avian and bovine blood, and further evidence of its association with birds is given by Mr. W. Ruttledge, who obtained specimens which were flying round pigeons, and by Mr. E. Hargreaves, who took engorged females from fowls.

GOLD COAST. Veterinary Laboratory, September, 1937.

NIGERIA. Ibi, October 1st–4th, 1911, J. Mc.F. Pollard.

SIERRA LEONE. Njala, April 17th, 1927, E. Hargreaves.

SUDAN. Dongola Province, Selim, February 10th, 1910, H. H. King; Um Inderaba Kord, January 5th, 1929, H. W. Bedford; Shambat, February 13th, 1929, H. W. Bedford.

Simulium hirsutum Pomeroy

The male, female and respiratory organ of the pupa were first described by Pomeroy (1922); the adult of both sexes were redescribed by the present writer (1936*a*), who also dealt with the larva and pupa.

GOLD COAST. Veterinary Laboratory, September, 1937.

SIERRA LEONE. Freetown, November, 1925, D. B. Blacklock.

TANGANYIKA. Morogoro, November 21st, 1917, A. W. J. Pomeroy; Amani, Sigi River, October 14th, 1917, C. B. Williams.

Simulium impukane De Meillon

In view of the fact that priority to the name *violaceum* must be credited to Enderlein's *Simulium*, it is necessary to find another name for the African species. Recently De Meillon (1936) described *S. impukane*. His figures of the male terminalia and respiratory organ of the pupa leave no doubt that it is actually the African *violaceum* of Pomeroy. The insect should therefore be known in future as *S. impukane* De Meillon.

NIGERIA. Ibadan, December 10th, 1910, A. W. J. Pomeroy.

NYASALAND. Zomba, February, 1913, H. S. Stannus.

Simulium lepidum De Meillon

De Meillon (1935) based his original description of the male and female on material from the Transvaal. The insect is widely distributed in Uganda (Gibbins, 1937*a*) and is now recorded from Tanganyika and the Cape.

CAPE. Mitchell's Pass, Ceres, December, 1934, H. G. Wood and C. W. Thorne.

TANGANYIKA. New Langenburg, October, 1917, H. S. Stannus.

Simulium medusaeformis Pomeroy

Pomeroy's figure (1920) of the male terminalia of this species depicts the clasper with a pair of terminal teeth. This character, which does not appear to be variable in *Simulium*, together with certain smaller differences, suggested that *S. ugandae* Gibbins was distinct. An examination of the type, however, shows a discrepancy in Pomeroy's drawing, as the clasper is armed with a single tooth. The female of *ugandae* also shows the extremely long gonapophyses which is characteristic of *medusaeformis*. The species should therefore become a synonym of *S. medusaeformis* Pomeroy.

Among the material from the South African Museum were two type females of *S. capensis* Enderlein (1935). A careful study of these specimens showed them to be indistinguishable from *medusaeformis*.

In view of these studies, it would appear extremely doubtful if *S. pseudomedusaeformis* described and figured by De Meillon (1936) is distinct, and it is suggested that it should be regarded as a synonym of *S. medusaeformis*.

CAMEROONS. Bangan, February 14th, 1916, A. W. J. Pomeroy.

CAPE. Cape Peninsula : Cape Town, Table Mountain, 3,000 ft., February, 1917 ; Glencairn, August 16th, 1932 ; Kalk Bay, Silvermine Stream, December 16th, 1932. French Hoek Mountains ; Gudo Pass, north of Ceres ; Wellington Mountains, Cedarbergen, 4,000–5,000 ft. All collected by K. H. Barnard.

NATAL. Weenen, May, 1924, H. P. Thomasset.

NYASALAND. Zomba, 1914, H. S. Stannus ; Zomba, Mponda Stream, E. Ballard.

Simulium neavei Roubaud

The male and early stages of this important species are still unknown. There have been many records of *S. neavei* biting man, but the observation made by Pomeroy of the fly attacking a mule at Amani in Tanganyika is the first record of the insect biting an animal. The specimens taken by Mr. R. E. Lloyd in Northern Rhodesia were captured in the act of biting man.

In his paper on the Ethiopian Simuliidae, De Meillon (1930) commented on the close resemblance of *S. vorax* Pomeroy to *S. neavei* Roubaud. The present writer has made a careful study of Pomeroy's material and finds no justification for regarding *vorax* as distinct from *Simulium neavei*.

KENYA. Kakamega Forest, River Yala, 4,800–5,000 ft., May 21st–29th, 1911, S. A. Neave ; Lumbwa Reserve, Chemosit River District, April, 1920, F. W. Dry.

NATAL. Weenen, May, 1924, H. P. Thomasset.

NORTHERN RHODESIA. River Kaomba, Serenje Boma, 4,200 ft., April, 1930, R. E. Lloyd.

NYASALAND. Mount Mlanje, January 20th, 1914, S. A. Neave.

TANGANYIKA. Amani, April 30th, 1914 ; Amani, December 14th, 1917, A. W. J. Pomeroy.

UGANDA. Western Ankole, 4,500–5,000 ft., October 10th–14th, 1911, S. A. Neave.

Simulium neireti Roubaud

The female was described by Roubaud in 1905. It is somewhat surprising to find that there is no record of the biting habits of this important human blood-sucking species, which is very troublesome in Madagascar. The writer cannot do better than quote the notes of two collectors who have been impressed

by the bite of the fly. J. J. Lloyd writes: 'The fly is well known all over Madagascar and seems universally distributed over the higher altitudes in the vicinity of forest-bordered streams. The native name is "Moka-foy," and the bite irritates but is not very painful and usually leaves a small hard lump immediately round the puncture. The insect is much more plentiful at an altitude of 4,000 feet than at the lower altitudes, and is sometimes present in such numbers as to settle on the back of the hand to the number of 20 at a time. While not perhaps killing stock, the fly keeps them in poor condition.' W. C. Holden states that 'the bite causes considerable irritation and swelling, which, however, completely disappears in a short time if the insect is allowed to withdraw blood; in this case there is no irritation. They often bite round the eyes and on the forehead under the helmet, in which case a spot of dry blood is the only thing noticed.'

The type of *S. neireti* is deposited in the National Museum of France, and, while it has not been possible to examine this specimen, Monsieur Séguy very kindly furnished two examples from the type locality, thus making it possible to confirm the identity of material in the British Museum.

MADAGASCAR. Ikiangara, 1904, Neiret; Andranolava, October 29th, 1907, J. J. Lloyd; Tsaratanana, 1,200–1,400 ft., November 28th, 1917, W. C. Holden.

Simulium nigritarsis Coquillett

Hitherto, considerable doubt has existed concerning the correct identity of the species, owing to the brief description of Coquillett (1901) and the absence of type specimens. The finding of a paratype among Pomeroy's material which has recently been presented to the British Museum now enables an accurate interpretation of the species to be made. Specimens from Nyasaland collected by R. C. Wood, and noted by De Meillon (1930) under *nigritarsis*, are now considered as *S. ruficorne* Macquart. The present study has confirmed De Meillon's (1934) subsequent interpretation of the species.

Careful examination of the type male and female of *S. caffrarica* Enderlein, kindly loaned for study from the South African Museum, has proved that it is no other than *S. nigritarsis* Coquillett.

CAPE. Cape Town, Peringney, 1915; Mossel Bay, R. E. Turner; Somerset East, October, 1930, R. E. Turner; Wellington, Hawequa Mountains (Seven Sisters), October, 1931, K. H. Barnard; Cape Town, Table Mountain, Platteklip Stream, December, 1934, and Clanwilliam, Cedar Mountains, October, 1934, K. H. Barnard; River Zonder End Mountains (Caledon Division), September, 1937, H. G. Wood and C. W. Thorne; Clanwilliam Division, Citrusdal, Warmbaths; Tulbagh, Great Winterhoek Mountains; Meiring's Poort, Zwartberg Range (north of Oudtshoorn); Caledon Division, Palmiet River; Worcester: Naudesberg, Langeberg Range and Rawsonville, du Toit's Kloof; Porterville, K. H. Barnard.

NATAL. Krantzkop: Weenen, 2,840 ft., July–December, 1923, January, 1924, February, 1925, November, 1927, H. P. Thomasset. Van Reenen, Drakensberg, December, 1926, R. E. Turner.

NYASALAND. Zomba, Mponda Stream, January 25th, 1913, E. Ballard; Zomba, H. S. Stannus.

Simulium palmeri Pomeroy

The male, female and respiratory organ of the pupa were described by Pomeroy (1922). Both *palmeri* and its close relative *blacklocki* have not been found elsewhere than in West Africa.

NIGERIA. Ubiaja, January 15th, 1921, A. W. J. Pomeroy.

SIERRA LEONE. River Sawa, D. B. Blacklock.

Simulium ruficorne Macquart

Simulium ruficorne is widely distributed* in Africa and also occurs on the island of Rodriguez.

The synonymy of this species was dealt with by Edwards (1934) and was adopted by the present writer (1936*a*). While a recent study of examples from widely separated countries has shown the presence of a light and a dark form, it is not intended to regard them as other than one species, particularly as the terminalia are identical and the difference appears to be mainly one of degree of colour.

Specimens from the type locality, Rodriguez Island, belong to the lighter form and are identical with those from Nyasaland, which differ from the rest in having much brighter yellow antennae; the dark band in the male formed by the apex of the second flagellar segment, which appears to be a characteristic of the species, is more conspicuous. The mesonotal pattern is almost identical in both sexes, and the broad brown median stripe is sometimes bisected by a fine line of silver scales. The female mesonotal pattern is identical in both forms, but in males of the darker form the lateral stripes are usually merged with the median stripe, posteriorly forming a large dark area. Examples of this species, previously known as *beckeri* and *divergens* from Algeria and Nigeria respectively, are lighter in colour than those from Uganda and Natal, but the respiratory organ of the pupa, which is characteristic in this species, is identical.

NATAL. Weenen, December, 1923, to December, 1924, H. P. Thomasset.

---

*Among a small collection of *Simulium* recently received from Palestine through the courtesy of Sir Guy A. K. Marshall, C.M.G., F.R.S., were two females of *Simulium ruficorne* Macquart, which were found to be identical with those from the type locality, Rodriguez Island. These interesting specimens, collected by Mr. J. Palmoni from Dagania A, Jordan Valley, 670 ft. below sea level, during 1935–36, present further evidence of the extremely wide distribution of the species. It is the first record of its occurrence in Palestine, though, as noted in this paper, *Simulium ruficorne* has previously been found within the Mediterranean region.

NYASALAND. Zomba, Mponda Stream, January 23rd–25th, 1913, E. Ballard; Cholo, 3,000 ft., September 16th, 1917, R. C. Wood; Luchenza, 2,000 ft., September 6th, 1918, R. C. Wood.

NIGERIA. Ibadan, December 4th, 1920, A. W. J. Pomeroy.

RODRIGUEZ ISLAND. August–November, 1918, H. J. Snell and H. P. Thomasset.

SIERRA LEONE. November, 1925, D. B. Blacklock; Freetown, Lumley, August 2nd-8th, 1926, R. M. Gordon.

SUDAN. Erhowit, May 25th, 1917, H. H. King.

Simulium simplex Gibbins

This species was originally described from Uganda by the present writer (1936*a*) in all its phases, with the exception of the egg. The undermentioned record of the species from the Cape, based on the character of the respiratory organ of the pupa, is therefore not without interest.

CAPE. Cape Peninsula, Glencairn, August 16th, 1932, K. H. Barnard.

Simulium speculiventre Enderlein

The female *speculiventre* is unlike any other Ethiopian Simuliid in that it has a narrow front and claws, with the basal projection absent from all but the hind legs.

SEYCHELLES. Mahe, 1908–9, Seychelles Expedition.

Simulium touffeum Gibbins

This species, which was hitherto only known to occur in Uganda, from which country the male, female, pupa and cocoon were recently described (Gibbins, 1937*a*), is shown by the undermentioned record to have a wider distribution.

NYASALAND. Zomba, Mponda Stream, E. Ballard.

Simulium tentaculum Gibbins

The original description of the male, female, larva, pupa and cocoon was made from Uganda specimens (Gibbins, 1936*a*). The species has since been recorded from the Belgian Congo (Gibbins, 1936*b*), and the undermentioned record from the Cape is based on the respiratory organ of the pupa.

CAPE. Cape Town, Table Mountain, Orange Kloof, March 11th, 1934, K. H. Barnard.

Simulium unicornutum Pomeroy

The species was first described by Pomeroy (1920) on the peculiar character of the respiratory organ of the pupa. Subsequently (1922) the same author gave an account of the adult of both sexes, which were redescribed by

the present writer (1936*a*), when a description of the larva, pupa and cocoon was also included.

SIERRA LEONE. Freetown, October, 1925, D. B. Blacklock; Njia, January, 1926, D. B. Blacklock.

UGANDA. West Nile, October, 1925, G. D. H. Carpenter.

Simulium wellmanni Roubaud

Only the female of *S. wellmanni* is known; it was described by Roubaud in 1906. An account of the blood-sucking habits of the insect was given by Austen (1909).

ANGOLA. Bihe, ' Bulu-Bulu ' Plain, April, 1905, F. C. Wellman.

BELGIAN CONGO. Mutambala, 1,950 m., October 23rd, 1925, J. Schwetz.

Simulium woodi De Meillon

As remarked on by De Meillon (1930), the female may easily be distinguished by the dark abdominal band comprising the fourth tergite. The male and early stages are unknown.

NYASALAND. Cholo, 2,700–3,000 ft., October, 1917, and November 26th, 1920, R. C. Wood.

REFERENCES

AUSTEN, E. E. (1909). Illustrations of African blood-sucking flies. p. 30. Lond.

BECKER, T. (1903). Aegyptische Dipteren. *Mitt. Zool. Mus. Berlin*, **2** (Hft. 3), 78.

COQUILLETT, D. W. (1901). New Diptera from southern Africa. *Proc. U.S. Nat. Mus.*, **24**, 27.

DE MEILLON, B. (1930). On the Ethiopian Simuliidae. *Bull. Ent. Res.*, **21**, 185.

——— (1934). Studies on insects of medical importance in South Africa. pt. I. *Publ. S. Afr. Inst. Med. Res.*, **6**, 253.

——— (1935). Studies on insects of medical importance in South Africa. pt. II. *Ibid.*, **6**, 323.

——— (1936). Studies on insects of medical importance in South Africa. pt. III. *Ibid.*, **7**, 208.

EDWARDS, F. W. (1934). The Simuliidae (Diptera) of Java and Sumatra. *Arch. Hydrobiol.*, Suppl.-Bd. **13**, ' Tropische Binnengewässer,' **5**, 137.

ENDERLEIN, G. (1935). Neue Simuliiden, besonders aus Afrika. *S.B. Ges. Naturf. Fr. Berlin*, 358.

GIBBINS, E. G. (1933). Studies on Ethiopian Simuliidae: *Simulium damnosum*, Theo. *Trans. Roy. Ent. Soc. Lond.*, **81**, 37.

——— (1934). Further studies on Ethiopian Simuliidae. *Ibid.*, **82**, 51.

——— (1935). *Simulium griseicolle* Becker, from the Sudan. *Ann. Trop. Med. & Parasitol.*, **29**, 177.

——— (1936*a*). Uganda Simuliidae. *Trans. Roy. Ent. Soc. Lond.*, **85**, 217.

——— (1936*b*). Congo Simuliidae. *Ann. Trop. Med. & Parasitol.*, **30**, 133.

——— (1937*a*). Simuliidae of the Buganda, Eastern and Western Provinces of Uganda. *Bull. Ent. Res.*, **28**, 289.

——— (1937*b*). Notes on Ethiopian Simuliidae. I. *Ann. Trop. Med. & Parasitol.*, **31**, 299.

POMEROY, A. W. J. (1920). New species of African Simuliidae. *Ann. & Mag. Nat. Hist., 8th ser.*, **6**, 72.

——— (1922). New species of African Simuliidae and further studies on the early stages. *Bull. Ent. Res.*, **12**, 457.

ROUBAUD, E. (1905). Les Mouka-Fouhi, Simulies nouvelles de Madagascar. *Bull. Mus. Hist. Nat., Paris.*, **11**, 425.

——— (1906). Insectes diptères: Simulies nouvelles ou peu connues. *Ibid.*, **12**, 519.

AN UNUSUAL STRAIN OF RABIES VIRUS IN A VAMPIRE BAT

BY

J. L. PAWAN, M.B.E., M.B., CH.B. (EDIN.), D.P.H.

(*Government Bacteriologist, Trinidad, B.W.I.*)

(*Received for publication February 15th*, 1938)

Recent experiences in Brazil, Trinidad, French Equatorial Africa, South Africa and elsewhere have shown that there is need for revision of our conceptions of the mode of transmission and of the clinical manifestations of rabies in both human beings and animals. The agents of spread are not members of the canine tribe only, but include the vampire bat, the meerkat, the mongoose, etc. ; while in man the classical symptoms of hydrophobia may be absent, and the disease may assume the form of a spreading myelitis much more frequently than was formerly supposed. The existence of unsual forms of rabies in animals in various parts of the tropics, as pointed out by Curasson (1936), and the varied properties of strains of the virus isolated in both Europe and Africa, tend to confirm the opinion of Marie (1927) and others that there is a plurality of both street virus and *virus fixe*. The two main characteristics of *virus fixe* are the absence of Negri bodies and the short and definite incubation period of 6 to 10 days when inoculated intracerebrally into rabbits. On the other hand, the presence of Negri bodies in street virus is considered to be so constant that in some centres its absence in a suspected animal contra-indicates infection with rabies, despite the fact that strains have been isolated in nature from animals in which the most careful search failed to reveal their presence. The other feature of street virus—its prolonged incubation—is an important characteristic ; when inoculated into rabbits death does not take place before 15 to 30 days. The short incubation period of four days for the Koritschoner (1923) and three days for the Jonnesco (1932) strains has therefore been the subject of much comment. But the literature available locally shows no record of a strain with such an abnormally short incubation as the one here described.

On May 14th, 1935, a *Desmodus* bat was caught at Diego Martin, Trinidad, and placed in solitary confinement until November 30th, when it was caged with with a rabbit, rb. 5. The rabbit was bitten on the tips of both ears during the night of November 30th–December 1st, and again on the left ear some time between 9 a.m. and 11.30 a.m. during the day of December 1st. It was then removed and kept under observation. On the 5th, four days later, it showed paralysis, and in the afternoon of the same day it died. The brain was removed and preserved in sterile glycerine saline, and the hippocampus, fixed in Zenker and stained by Mann's method, showed oxyphilic cytoplasmic inclusions, which may have been Negri bodies, but their differentiation was not clear. On the

following day, December 6th, two rabbits were injected with a brain emulsion of rabbit rb. 5, one intra-ocularly and the other intrasciatically. On the 8th, they showed paresis, one in the left forelimb and the other in both hind limbs, and in the afternoon of the same day they died ; but Negri bodies could not be seen. On the 15th, two other rabbits were again injected with a brain emulsion from the original rabbit rb.5, one intra-ocularly and the other intra-sciatically. Again, both rabbits died in two days' time, i.e., on the 17th, but Negri bodies were not seen.

On February 6th, brain material from rabbit rb. 5, which had been lying in glycerine saline for two months, was injected intracerebrally into rabbit 59. Paresis of the right forelimb appeared on the 8th, and death took place on the same day, i.e., two days after inoculation. On the 19th, material from rabbit 59, which had been in glycerine saline for 11 days, was injected at 11.30 a.m. intracerebrally into rabbit 70. At 4 p.m. on the same day, the rabbit was seen to be apathetic and drowsy, and in the afternoon of the 20th it was found dead. The brain of rabbit 70 was removed and kept in glycerine saline for 13 days, and on March 3rd it was injected intracerebrally into rabbit 78. Again, death took place in two days, but no Negri bodies could be seen. The process of intracerebral inoculation was repeated three times (rabbits 79, 80, 81), and in every instance death with paralysis followed in two days. On two occasions cultures from the brains of the rabbits were made in broth and glucose agar (stabs), but no organism was grown, nor could any organism be isolated in blood cultures, while post-mortem examinations showed no gross lesions. On March 20th, a brain emulsion of rabbit 81 was injected *sub-cutaneously* into rabbit 82. Again death followed in two days. On March 27th, an emulsion of the left sciatic nerve of rabbit 82 was injected into the right sciatic nerve of rabbit 106. Death in this case took place in 20 days, i.e., on April 18th, and Negri bodies were present in the hippocampus. On the same day, the left sciatic nerve of rabbit 106 was injected into the right sciatic nerve of another rabbit, 121. Death took place on May 1st, i.e., in 13 days, and numerous Negri bodies were seen in hippocampus. On November 4th, a brain emulsion of rabbit 121, which had been in glycerine saline for 6 months, was injected intracerebrally into rabbit 1a. Death with paralysis took place again in two days, on the 6th, and a few Negri bodies were seen in the hippo-campus.

COMMENTS

The proneness to regard as the aetiological agent a micro-organism or virus found associated with a diseased condition in experimental animals has led to disastrous results in the past. Koch's postulates are applicable to ' micro-scopic ' organisms, but the difficulty of growing viruses apart from their host or tissue cells, and the uncertainty of their serological reactions, warrant caution in the interpretation of experimental results. Experiences with the strepto-

coccus in poliomyelitis, the virus of herpes in epidemic encephalitis, the varicella virus in rabbits, the encephalitis of experimental rabbits and encephalo-myelitis of man, etc., have shown how a fortuitous, or co-existing, infection, or the activation of a latent natural infection by an experiment, may suggest erroneous deductions. In the case of the virus here studied, it is doubtful whether the inclusions seen in the first experimental rabbit, the one bitten by the bat, were really Negri bodies, though their ill-defined appearance may have been due to the rapid course of the disease and the consequent lack of time for their complete development. But the constancy of the symptoms of infection with death, and the ultimate development of Negri bodies by intrasciatic injections, prove that one and the same virus, that of rabies, was responsible for the results, unless one postulates that in the original bat or the experimental rabbit there existed, coincidentally with rabies virus, an unknown infection capable of producing paralysis with death in rabbits in two days. Remlinger and Bailly (1931) pointed out the similarities between rabies virus and other neurotropic viruses, and indicated the methods by which differentiation should be undertaken ; but the serum neutralization and cross-immunity tests are tedious and uncertain. Recent demonstration by Nicolau and Kopciowska (1935) that the negrigenic power of *virus fixe* can be restored by repeated inoculations through the sciatic nerve should therefore offer a ready and reliable means of identifying rabies. In this instance, it was possible to confirm their findings, for intrasciatic infection after a prolonged incubation produced death with the development of Negri bodies. It may, however, be mentioned here that, in another study, failure to produce Negri bodies after several intrasciatic inoculations followed the use of a local strain of *virus fixe.* A point of further interest is the fact that three different routes of inoculation produced death in two days, and, as was to be expected with such a short incubation, Negri bodies could not be seen. After the virus had been habituated to intracerebral growth, the incubation period with intrasciatic injections was lengthened to 20 and 21 days, but Negri bodies were present.

The rapid spread of infection with death in two days is evidence of the exalted virulence of this virus, and recalls to mind the suitability of the description of Nicolau and his co-workers (1933) that infection with rabies virus is a ' septinévrite.'

SUMMARY

1. A strain of rabies virus capable of causing death in rabbits in the abnormally short period of two days was found in a vampire bat in Trinidad.

2. Cold storage for six months in glycerine saline did not destroy either its virulence or its power to cause death in two days.

3. The possibility of infection with a coincidental and unknown virus cannot be definitely excluded.

4. The negrigenic power of this virus can be restored by intrasciatic inoculations.

REFERENCES

CURASSON, G. (1936). Traité de pathologie exotique vétérinaire et comparée, **1**, 520.

JONNESCO, D. (1932). Recherches sur un virus rabique de rue à virulence renforcée. *Ann. Inst. Pasteur*, **49**, 435.

KORITSCHONER, R. (1923). Quoted in Kraus, Gerlach and Schweinburg : Lyssa bei Mensch und Tier, p. 49. Vienna, 1926.

MARIE, A. C. (1927). Sur la nature du virus rabique. *Ann. Inst. Pasteur, Suppl.* : *Conférence Internationale de la Rage*, p. 13.

NICOLAU, S., and KOPCIOWSKA, L. (1935). Essais de transformation du virus rabique fixe en virus des rues. *Ibid., Suppl.* : *Numéro Commémoratif sur la Rage*, p. 108.

——— MATHIS, C., and CONSTANTINESCO, V. (1933). La rage autochtone (maladie du chien fou) en Afrique occidentale française. *Ibid.*, **50**, 778.

REMLINGER, P., and BAILLY, J. (1931). Les confusions entre les virus neurotropes et le virus rabique : moyens de les éviter. *Bull. Inst. Pasteur*, **29**, 9.

PROTECTION FOR THE ROOFS OF BUILDINGS IN THE TROPICS

BY

D. B. BLACKLOCK

(*From the Department of Tropical Hygiene, Liverpool School of Tropical Medicine*)

(*Received for publication March 10th*, 1938)

In recent years there has been a great extension in the use of such materials as corrugated iron for roofing houses in the tropics. There is no doubt that the substitution of this kind of roof has several advantages over the thatch, whether thick or thin, which was formerly so greatly used.

One of the drawbacks of thick thatch is the good lodgement which it provides for rats, and the increase thereby of the danger of plague, typhus and other diseases. Another serious drawback is the risk of fire. Great loss and damage may occur if the roof takes fire, and, in a strong wind, not only the house itself but all the neighbouring ones are likely to be destroyed. A thin thatch, while not affording shelter for the lodgement or breeding of rats, and not likely to cause such serious damage by fire, has the drawback of not providing the rain-proofness of thick thatch.

Corrugated iron is more satisfactory in these respects than either thin or thick thatch. But one very serious disadvantage of corrugated iron, when exposed to the sun, is the great heat generated in rooms below it ; a less serious one is the irritating noise made by the fall of heavy rain upon it.

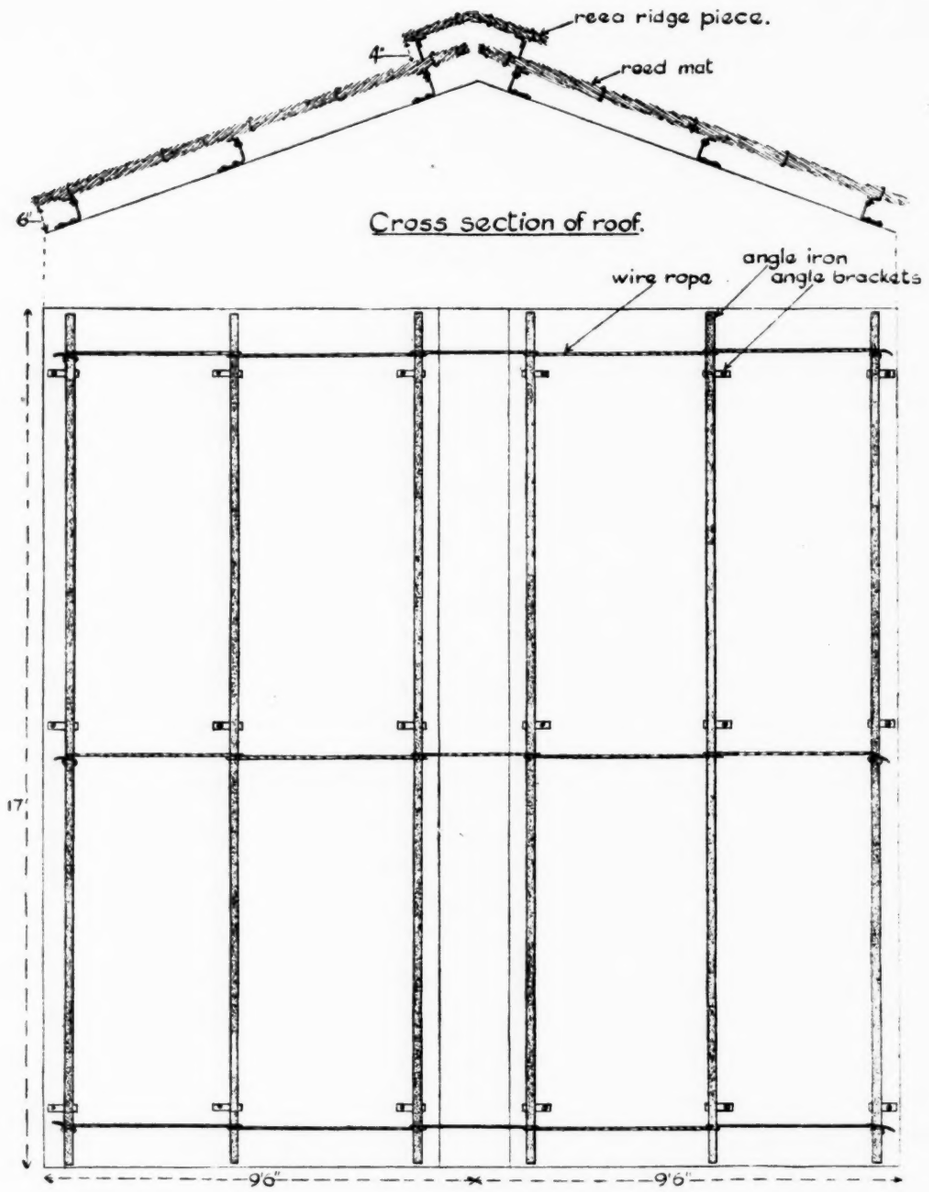
In an address on housing, delivered before the Tropical Hygiene Section of the Health Congress of the Royal Sanitary Institute at Birmingham in 1937, I drew attention to a method by which the disadvantages of the iron roof, especially the excessive heat and the noise, might be minimized by the use of thatch, without introducing at the same time the danger of rat-lodgement and fire.

As stated in that address, the idea of the method was derived from houses seen many years ago at Daru, the military station of the Royal West African Frontier Force in Sierra Leone. In that station all-metal houses were erected on concrete floors, and over them high and wide roofs were built of poles and thick thatch. This kind of house was very cool, and débris or rain falling from the thatch were intercepted by the metal roof.

The modified arrangement for the protection of the metal roof, mentioned and illustrated by photographs at that meeting, is described here in greater detail, in response to several requests for further information.

The metal roof, which is covered, measures 9 feet 6 inches from the eaves to the ridge, and is 17 feet long.

The principle is to place over the roof, but not in actual contact with it, a thin but strong layer of thatch, sufficient to prevent the sun's rays from striking the metal roof. The space between the roof and the thatch is fixed arbitrarily at 6 inches, but it may be found that a greater space gives even better results. The aim in selecting a distance of 6 inches is to ensure not only good air-space



and free ventilation between the metal roof and the thatch matting, but also to produce a space which can be kept clear of obstruction and in which rats cannot lodge. It is possible to pass a long stick freely all over the roof between the mat and the metal, and to clear out any obstruction. If a small space of only an inch or

two had been retained, these advantages would probably have been much diminished.

Norfolk reed matting is employed as the thatch. The matting is manufactured, to the dimensions required, by a firm which specializes in thatching houses with Norfolk reed material. The thickness of the matting selected is 2 inches ; the mat is made by tying reeds of the required length in bundles of about 2 inches in diameter, by means of strong tarred string at 2-feet intervals. These bundles are then tied to each other by strings at similar intervals, so as to form a stout mat which can be rolled up.

For the attachment of the matting to the roof, a simple angle-iron framework, fixed on supporting brackets, is used. The brackets are right-angled, arranged with one limb projecting upwards far enough to let the framework be attached to it by bolts and nuts, and to leave the required space of 6 inches above the metal roof. The other limb is bolted securely on to the convexity of a corrugation of the roof metal. Eighteen of these brackets are employed, nine on each side of the roof, arranged in three rows of three.

The angle-iron is $1\frac{1}{4}$ inches, fixed to the brackets, three stretchers, each of 17 feet long, being applied to each side of the roof. As there was no more angle-iron available at the time of construction, additional points for the attachment of the matting were provided in this case by the use of ordinary twisted-wire clothes-line. The wire-line was attached firmly to the angle-iron frame and stretched across the spaces formed by it.

The frame having been completed, the matting is rapidly fixed to it by loops of stout tarred string passed through the mat from the outside and attached securely to the framework. At intervals, loops of galvanized iron wire are employed to give additional security. An important point in applying the matting is to leave at the ridge a slight separation between the tops of the two opposite sets of mats. This is to ensure a free circulation of air over the metal roof and out above the ridge. If it is thought necessary to prevent the strip of ridge being exposed to the sun or to prevent rain passing in here, a ridge-piece can be made and put in position, again taking care to leave an air-space between it and the matting. A ridge-piece of 1 foot 6 inches wide, fixed 4 inches above the thatch, should suffice to keep the sun off the small line of ridge exposed, and to keep out the rain.

The reed matting is composed of the same type of reed as is used widely for thatching houses. In the form of thick thatch applied directly to the roofs it is extremely durable. It is anticipated that the matting, although exposed to somewhat harder usage, will still have a long life. Its appearance improves as a result of weathering. It is interesting to note that Messrs. Farman, who specialize in Norfolk reed thatching, claim that this thatch will give about a hundred years' service, as proved in many instances. They claim also that it is quite impervious to vermin. This question of vermin is of particular mportance in the tropics.

More important, however, for our present purpose, is their statement that ' the even temperature which is maintained under thatch is beneficial to horse-boxes and racing-stables, as seen in the neighbourhood of Newmarket, Coventry and other places.'

With regard to the tying and fixing of the mats, the methods adopted have proved adequate. The roof-covering has been subjected, during a year since its construction, to extremely severe gales, as it is situated in a very exposed position ; it has withstood these winds quite successfully and remained intact.

The cost of materials used is given below :—

	£	s.	d.
Norfolk reed mats	8	14	0
Angle-iron, $1\frac{1}{4}$ inches, 102 feet at $1\frac{1}{4}$d. per foot	0	10	$7\frac{1}{2}$
Angle-brackets, 18	0	3	0
Metal clothes-line, 120 feet	0	1	$10\frac{1}{2}$
Bolts and nuts	0	1	0
	£9	10	6

It will be noted that the reed matting is the only expensive item. Probably some form of elephant-grass or local reed will prove quite satisfactory. Materials of this kind can be obtained in most parts of the tropics for the cost of cutting and transporting them.

Dr. Davey in Sierra Leone is at present engaged in carrying out experiments, using local materials, to ascertain the temperature-effects produced by insulating metal roofs from the sun's rays by this method, under tropical conditions. With regard to the danger of fire, the thinness of the layer of thatch and its separation from the structure of the house are minimizing factors ; another is that the matting can easily be treated with fire-proofing solution, if thought advisable.

Professor J. McLean Thompson has been good enough to provide the following note on the thatch material.

The specific name is *Phragmites arundinacea*. It has a preference for mud, upon which it has a slight binding effect, especially in quiet lagoons or rather stagnant estuaries. It cannot survive drying of the soil nor complete submersion, but it thrives upon rhythmic partial submersion for 1 or 2 feet of its main height.

OBSERVATIONS ON THE RESPIRATORY METABOLISM OF MALARIA PARASITES AND TRYPANOSOMES

BY

SIR S. RICKARD CHRISTOPHERS*

AND

J. D. FULTON†

(*London School of Hygiene and Tropical Medicine*)

(*Received for publication January 27th*, 1938)

INTRODUCTION

The measurement of O_2 uptake has been extensively used recently in the study of metabolism of different organisms, of body tissues, and of the nature of the enzymes upon which this largely depends. We have made this method the basis of investigations upon malaria parasites and trypanosomes in relation to chemotherapeutic effect. Quinine and some related substances have long been known to have the power of inhibiting certain enzymes, and it appeared to us possible that it might be through such a property that these drugs exert their effect chemotherapeutically, thus accounting for their action in the very low concentrations in which they are present in the body. It has been suggested, for example, by Dixon (1937) that therapeutic effect may be brought about in some cases by combination of the drug with the cytochrome or other hydrogen-carrier of the cell, thus inhibiting cellular respiration. The present paper deals with such part of our researches as relate to the normal metabolism of the above-mentioned organisms. Observations upon the inhibitive effect of a number of compounds are given by us in a second communication (see below, p. 77).

It is obvious that a study of the normal metabolism is no less an integral part of our investigations than are the observations relating more specifically to the inhibitive action of the different drugs. Without a knowledge of the normal respiratory mechanism of the organism dealt with, little progress could be made in understanding the action of drugs working therapeutically or inhibiting *in vitro* through this mechanism. Such study was the more necessary since even with the respiratory metabolism of trypanosomes there is field for further enquiry, whilst the respiratory mechanism of malaria parasites is up to the present, so far as we are aware, entirely unknown.

The organisms used by us were a normal and arsenic-fast strain of *Trypanosoma rhodesiense*, kindly supplied by Professor Yorke and sub-cultured by passage in mice, and a strain of *Plasmodium knowlesi*, obtained through the

* Leverhulme Fellow under the Medical Research Council.

† Beit Memorial Fellow.

kindness of the Director of the Malaria Survey of India, and maintained for several years in this laboratory in *Macacus rhesus* monkeys.

It was convenient for our work to carry out observations more or less at the same time upon the two organisms, and, since such parallel observations served especially to emphasize the marked differences in the respiratory mechanism in the two cases, we have adopted a similar method of presentation in our paper. Observations upon the more readily obtained and better-known trypanosome material are followed by more or less parallel work upon malaria parasite substance, the metabolism of which in relation to chemotherapy may be said to have been our main object.

For measuring O_2 uptake we have employed a set of six Barcroft differential manometers working in a constant-temperature bath at 37° C. Three ml. of ' mixture ' was used in the right-hand flasks, with 3·0 ml. of ' control mixture ' in the left-hand flasks, the ' control mixture ' being exactly similar to the ' mixture ' except for the presence of organisms in the right-hand flask; 0·5 ml. of 20 per cent. potash with rolled special filter-paper was used in both cups. The taps were closed after allowing 15 minutes' shaking for equilibration, and readings were made at periods of 15 minutes for one or two hours, according to circumstances, the uptake during each period of 15 minutes, as shown by difference, being that usually given in the text. In many cases, and especially where the result was in any way critical, the mean of three manometer readings was used with a similar number for the control, but the concordance of results allowed us in many cases to cut the number down to two, with consequent gain in time. In any cases of doubt, one or more repeat experiments were carried out.

Some of our results for the normal strain of trypanosomes have been based on very large numbers of observations carried out over considerable periods of time, since the information required was given by normal controls for over 400 examinations made in connection with the inhibitive action of drugs recorded elsewhere. Other values, e.g., the respiratory quotient for parasite substance, where a good deal depended upon the actual figures obtained, have been repeated until we felt satisfied that the values given were reliable.

Experiments with red cells and serum show that in both cases there is a small actual or apparent uptake as shown by the manometric readings. The following are representative examples given in μl. per successive intervals of 15 minutes. The red cells were put up as suspension in serum, of which an equivalent quantity was present in the opposite flask.

It is possible that the small uptake by red cells is due to some additional oxygenation of the haemoglobin as a result of the shaking, or to a small actual respiratory uptake, as with nucleated cells but in smaller degree.

In regard to the serum, the apparent uptake is considered to be due to CO_2 driven off by shaking at 37°. This, on absorption by the KOH, would appear as O_2 uptake. That such CO_2 is given off is indicated by the pH relationships

TABLE I

	r.b.c. deposit		Serum	
Source	Normal monkey	Normal monkey	Normal monkey	Heavily infected monkey
Amount present in flask in ml.	0·25	0·75	1·0	1·0
15 minutes	6	13	11	12
30 „	3	6	6	8
45 „	0	3	4	3
60 „	3	3	2	3
75 „	0	1	0	1
90 „	0	1	2	0
105 „	4	2	1	1
120 „			0	0
Total uptake	16	29	26	28

mentioned in a later section.

The small uptakes noted in no way compare with the much larger readings given by parasite substance or trypanosomes, and, except in special cases, may be neglected. Any inaccuracy from serum is eliminated in our experiments, since in all cases a similar quantity is always contained in the control (left) flask.

OBSERVATIONS ON *TRYPANOSOMA RHODESIENSE*

Work on the respiratory metabolism of trypanosomes has engaged the attention of a number of authors. Nauss and Yorke (1911) noted the loss of oxygen from trypanosome-infected blood, and observed that the carbon dioxide increase did not correspond with this loss. Later, Yorke and others measured the amount of sugar consumed by trypanosomes *in vitro* and found that it was enormous, while Biot, Biot and Richard (1911) had already indicated the importance of glucose for these parasites. Fenyvessy and Reiner (1924) measured the oxygen uptake and CO_2 production for *T. equiperdum*, and Fenyvessy (1926) showed that acid is produced by these organisms in sugar solutions. Fenyvessy and Reiner (1928) showed also that oxidative as well as glycolytic processes played a part in the breakdown of sugar. Kudicke and Evers (1924) investigated a large number of sugars with regard to the power of supporting life in trypanosomes, and found that galactose was the only common sugar which failed to do so satisfactorily. Of a number of other substances investigated, glycerol was found to support life. Reiner, Smythe and Pedlow (1936) studied the mechanism of decomposition of glucose by *T. equiperdum* and *T. lewisi*, and established quantitative relationship between oxygen uptake,

glucose consumption and acid production. The chief products obtained were glycerol anaerobically and pyruvic acid aerobically.

Recently Jancsó and Jancsó (1935) have observed a reduction in the number of trypanosomes in infected animals treated with synthalin (decamethylene diguanidine), which these authors ascribed mainly to hypoglycaemia following effect of the drug upon the organs concerned with sugar metabolism. Lourie and Yorke (1937) have shown, however, that synthalin has a direct action upon the parasites.

Material and Methods

In all except special cases, the 3·0 ml. of ' mixture ' in the experimental (right) flask consisted of 1·0 ml. ' standard suspension,' 1·0 ml. of Ringer's fluid made up to contain 0·6 per cent. glucose, thus giving a final 0·2 per cent. in the mixture, and 1·0 ml. of M/15 phosphate buffer pH 7·4. The control (left) flask mixture was identical, except that the 1·0 ml. ' standard suspension ' was replaced by 1 ml. of the same serum as used in making the suspension.

The following method was used by us in the preparation of ' standard suspension.' On the third day of infection, following a suitable (intraperitoneal) injection of infective material when the mouse blood is swarming with trypanosomes, the animal is killed with chloroform and the chest opened. Blood, usually from four to six mice, is withdrawn by cardiac puncture and centrifuged in about an equal volume of 2 per cent. citrate containing 0·1 per cent. glucose at 1,700 r.p.m. for 10 minutes. A suitable quantity of the upper white layer consisting of trypanosomes is removed by pipette and centrifuged for a further 5 minutes at the same speed in a narrow graduated tube holding about 1·0 ml.; 0·3 ml. of the deposit so obtained is removed and mixed with 8 ml. of rabbit serum. This forms the ' standard ' trypanosome suspension, containing 1 in 28 approximately of deposit suspended in rabbit serum, or 0·036 ml. of deposit in the 1·0 ml. added to the flask. The ' suitable quantity ' of deposit referred to is that which, taken from the first centrifuging of citrated mouse blood, was judged to give approximately a constant length of column after the second centrifuging, e.g., 0·5 ml. The top 0·3 ml. was then taken for the suspension. This precaution is necessary, as the amount of trypanosome substance in a given volume of deposit depends not only on the speed and time of centrifuging, but on the length of the column of deposit also. With these precautions, and allowing for occasional chance departures or variations in the quality of the material collected (see below), the uptake from a given quantity of suspension was very approximately constant (see Table II).

The rabbit serum used by us was not in our routine experiments inactivated by heat, and this may account in part for some variation in O_2 uptake through lethal effect on the trypanosomes in some cases. However, in some parallel experiments with untreated serum and with serum from the same animal inactivated by heating for half an hour at 56°, no significant difference in oxygen

uptake was noted. We have avoided the use of any infected mouse on the point of death (when convulsions start), since it appeared that in such cases, with low blood sugar content, rapid death of the trypanosomes might sometimes occur. Further, to avoid the possible lethal effects of low glucose content in the blood whilst obtaining material, we have used 0·1 per cent. glucose in the collecting citrate, as noted above.

The trypanosome content of the ' mixture ' made from such suspension was found, as the mean of a considerable number of determinations, to be 54,000 trypanosomes per μl. The standard suspension, therefore, contained $1{\cdot}62 \times 10^5$ trypanosomes per μl., whilst the number of trypanosomes in the flask of each Barcroft manometer (i.e., 1·0 ml. of suspension) was $1{\cdot}62 \times 10^8$.

When centrifuged at 2,000 r.p.m. for 45 minutes, 1·0 ml. of deposit, as used in making standard suspension, became compacted to 0·364 ml., and the dried weight of 1·0 ml. standard (uncompacted) deposit, less dry material in the fluid portion as estimated above, was 0·031 gm. The amount of dry trypanosome substance in 1·0 ml. of standard suspension was therefore 0·0011 gm., this being the amount present also in each flask of the Barcroft.

O_2 Uptake by Trypanosome Substance

The following data obtained from 53 determinations on normal trypanosomes and 27 on arsenic-fast trypanosomes, using the standard amount (0·036 ml.) of trypanosome substance with 0·2 per cent. glucose, indicate the amount of O_2 uptake under the conditions of our experiments. The preparations were the ' normal ' controls used when determining the inhibitive effect of drugs, the results of which are given by us in another paper. The observations have been grouped in the order of O_2 uptake in the first hour. They show the uptake in μl., as recorded for each 15 minutes up to the period of two hours.

As these results relate to ' standard ' amount, they clearly show a certain range of variation in the figures given by different preparations examined at various times over the period of our observations. The figures, however, include all our observations, a certain number of which even at the time were recognized to be in some way abnormal. Most of the extreme values are probably due to errors in technique. If four such outlying observations be excluded, the normal strain has shown 49 observations lying between 200 and 350 μl., within which range also lie 27 for the arsenic-fast strain, so that, considering the circumstances, the variation is not great. How far such variation has been due to differing amounts of trypanosome substance employed, or to differences in the material from a variety of causes, is not known. Where a lethal factor has operated during blood collections or as a result of mixing with a noxious rabbit serum, this would naturally be reflected in the number of living trypanosomes finally present, and might have influenced the recorded uptake. It is possible also that the uptake has been influenced to some extent by the state of the trypanosomes, protein content of the particular serum, etc.

TABLE II

Normal trypanosomes	O_2 uptake in 1 hour in μl.						Total number of observations	Mean uptake all classes
	150–	200–	250–	300–	350–	400–		
Number of observations	1	7	23	19	2	1		
Mean of O_2 uptake—μl. for 15-minute periods								
15	52	58	75	85	102	113	53	78
30	51	60	75	87	104	105	53	79
45	50	59	69	79	93	98	53	72
60	43	52	63	69	77	84	53	63
75	36	49	59	63	66	78	35	60
90	35	46	52	57	64	57	37	54
105	32	43	45	50			26	47
120	27	38	42	45			19	42

Arsenic-fast trypanosomes	O_2 uptake in 1 hour in μl.						Total number of observations	Mean uptake all classes
	150–	200–	250–	300–	350–	400–		
Number of observations		3	18	6				
Mean of O_2 uptake—μl. for 15-minute periods								
15		69	74	83			27	76
30		63	75	82			27	75
45		55	69	76			26	69
60		48	61	65			26	61
75		45	57	57			14	55
90		40	51	57			12	52
105		37	45	47			10	45
120		34	36	46			4	38

That the uptake was closely related to the number of trypanosomes is shown by the following counts in thousands per μl. in relation to the uptake by the respective mixtures in μl. O_2 in one hour.

Class μl. in 1 hour	Number of observations	Mean uptake in class	Mean count for class $\times 10^{-3}$	Uptake per 1,000 trypanosomes
200–	3	233	43·7	5·33
250–	9	284	52·4	5·42
300–	10	320	59·7	5·36
Total	22	293	54·5	5·37

From these data the uptake by 1,000 trypanosomes per μl. in the flask would appear to have been very constantly 5·37 μl. O_2 in the hour in those cases in which the trypanosomes were counted.

On the whole, we think that the O_2 uptake probably gives an indication of numbers of living trypanosomes as reliable as do counts very carefully carried out.

The results given in Table II show that in the presence of a sufficiency of glucose (0·2 per cent.) the mean O_2 uptake (during the first half hour) was at the rate of 0·314 ml. per hour for the amount of trypanosome substance used, i.e., for the normal strain 0·194 ml. per hour per 10^8 trypanosomes, 8·72 ml. per ml. of standard deposit, or 285 ml. per gm. dried substance per hour (Q_{O_2} = 285).

The observations clearly show that there is no significant difference in respect of O_2 uptake by ' normal ' and ' arsenic-fast ' strains.

The rate of uptake for the period noted (two hours) is approximately linear (see graph, p. 63). There is, however, a small though definite and increasing fall-off in uptake noticeable after the second period of 15 minutes, so that, in the last reading period, the figure is only approximately half that relating to the initial uptake. This reduction is not prevented by addition of a greater amount of of glucose (see Table V). It is considered to be due to acid formation (see later section).

The following data were obtained with varying quantities of trypanosome substance, the figures given being all reduced to ml. O_2 uptake per hour per ml. standard deposit.

TABLE III

Time in minutes	Amount in ml. of standard deposit used					
	0·036	0·024	0·018	0·009	0·0045	0·0022
15	8·67	9·17	8·89	9·33	4·44	1·82
30	9·00	9·33	7·78	7·56	6·22	5·45
45	8·56	8·67	8·22	6·67	8·00	5·45
60	7·44	7·67	8·00	8·00	8·00	5·45
75	6·67		8·00	6·67	8·00	5·45
90	6·00		7·78	7·56	8·89	9·09
105	5·56		6·89	7·56	9·78	14·55
120	5·00		5·33	6·67		

In general, the results are proportional to the amount of trypanosome deposit used. The falling off with time in O_2 uptake, already noted with standard suspension, becomes less marked with the smaller quantities, and may, in amounts 1/8 and 1/16 the standard, be replaced by an increase in O_2 uptake, following, however, an initial smaller proportionate uptake which has always been observed. Probably the fall is due to concentration of acid produced, and the increase to multiplication in the very dilute suspensions where the organisms are presumably under the most favourable conditions. Never-

theless, for practical purposes a suspension of 0·036 has advantages in giving a larger uptake, with consequent accuracy in measurement.

An experiment in which trypanosomes were suspended in serum alone, with the requisite amount of added glucose to give the same concentration as that in the normal serum-buffer-Ringer medium as control, showed that serum by itself as medium had no advantages over the other, the O_2 uptake being smaller over a period of one or two hours than that in the normal medium.

Relation of O_2 Uptake to Glucose

As already noted by other authors, by far the most important circumstance modifying O_2 uptake by trypanosomes is the presence or absence of glucose in the medium.

If only Ringer solution not containing glucose be used in making up the mixture, the O_2 uptake proceeds actively as a rule for about the first 15-minute period. Instead of continuing in a more or less linear manner, as already described, the graph during the next 15 minutes or so then becomes approximately horizontal (see graph). It is evident from the results of various experiments that uptake for a brief period at the beginning has been due to such amount of glucose as was contained in the serum used in making the mixture, and that when this was exhausted O_2 uptake ceased. Not only is this the case, but on examining such a preparation trypanosomes will be seen to move sluggishly, become motionless and rather rapidly undergo deformation and lysis. The effect of deprivation of sugar has, in fact, all the appearances of an active poisoning, as is seen in experiments with lethal drugs. This effect is shown equally with normal and with arsenic-fast strains.

Table IV

Showing effect of presence and absence of added glucose upon O_2 uptake of normal and arsenic-fast strains of trypanosomes

Time in minutes	Normal 0·036 ml. trypanosome substance		Arsenic-fast 0·03 ml. trypanosome substance	
	Glucose		Glucose	
	+	—	+	—
15	74	58	55	47
30	74	20	60	45
45	78	7	53	14
60	72	3	47	3
75	67	2	45	0
90	57	1	41	1
105	41	1		

The amount of glucose used up is dealt with later. It is approximately 0·1 per cent. per hour for the amount of suspension already noted.

We have found that for the period of two hours over which our experiments have been usually conducted any increase in glucose over 0·2 per cent. possessed no advantages, whilst addition of 0·1 per cent. only did not suffice for this period, as shown here in a comparative experiment determining this point. In all our experiments, except for special reasons, glucose to give 0·2 per cent. in the final mixture was therefore added.

TABLE V

Time in minutes	Per cent. of glucose added		
	0·1	0·2	0·3
	O_2 uptake in μl.		
15	83	83	79
30	87	91	81
45	84	83	73
60	79	79	71
75	65	67	62
90	16	62	57
105	4	59	61
120	0	52	43
135		34	36
150		25	21

In such tables, using up of available glucose is shown by an unmistakeable and rapid reduction at some point, e.g., at 90 minutes in the column giving readings for 0·1 per cent. glucose. A more gradual reduction in uptake, as previously noted, is seen as a result of acid formation, and is described later.

Such additional reduction, as seen in columns 3 and 4 of the table, is not affected by addition of glucose.

The using up of glucose is very strikingly shown in the case of infected mice. As these approach death, which is usually heralded by convulsions, determination of the blood sugar gives values well below that of a normal mouse, and estimation of the glycogen content of the livers of these animals indicates a much depleted store.

From these results it is seen that there is a steady drain on the store of liver glycogen as the infection proceeds. Of course, there is a reduction of liver glycogen in the case of starved animals ; but as a matter of fact it appears to us that during infection and till near death the animals behave quite normally, are active and eat well ; and the same facts have been noted by Fenyvessy (1926). Whether the fall in liver glycogen is due to attempted maintenance of

blood sugar level in conditions where the infecting parasites consume large quantities, or to failure to store glycogen, we are unable to say.

BLOOD SUGAR CHANGES

Condition of animal	Number of duplicate determinations	Range of blood sugars mg. per 100 ml.	Mean blood sugar mg .per 100 ml.
Normal (uninfected)	12	119—189	134
Heavily infected	6	29—48	36
Convulsive	1		3

CONTENT OF LIVER GLYCOGEN

Condition of animal	Number of animals	Mean blood sugar mg. per 100 ml.	Weight of livers	Glycogen per 100 gm. liver
Normal	12	127	19·6	1,194
Slight infection	5	100	8·2	1,084
Heavily infected (A) ...	13	32	21	185·4
Heavily infected (B) ...	12	27·5	22	177
Recently dead from infection	7		13·8	60·5
Fasted 24 hours	6	83	10	185·4
Fasted 9 hours	6	97	9·2	514

ACID PRODUCTION BY TRYPANOSOMES

As shown by Fenyvessy (1926) and by Reiner *et al.* (1936), the utilization by trypanosomes of glucose gives rise to the formation of acid.

The mixture after a Barcroft experiment was always found to have a considerably lower pH than when originally made up. This was at first usually about 7·6 (due to alkalinity of added serum), but, after two hours' shaking with normal O_2 uptake, it usually fell to 6·8 or less. That this striking drop in pH, due to acid formation, accounts for the gradual falling off in O_2 uptake already noted is suggested by the following experiment, in which buffer contained in the side arm was added during the course of oxygen uptake (Table VI).

That the falling off here is not dependent on lack of glucose is evident though, as shown in some of the columns, lack of glucose may come into effect if the experiment be continued beyond the period for which the glucose added has been suitable.

In this connection it is of interest to note that, if horse serum was used in place of rabbit serum, the initial O_2 uptake was not greatly different, but later the graph relating O_2 uptake with time was more linear and final total uptake was greater. This was found associated with a greater total buffering power of horse serum, as determined by electrometric titration. The results in Table VII illustrate the above point (see also graph, p. 63).

TABLE VI

O_2 uptake in μl. with and without addition of extra buffer and glucose

Time in minutes	No extra glucose added				0·2 per cent. extra glucose added	
	No buffer added	1·0 ml. original medium added	No buffer added	1·0 ml. buffer added	No buffer added	0·64 ml. buffer added
15	53	58	71	70	46	49
30	55	57	70	69	47	49
45	50	54	54	55	41	44
60	45	47*	51	52	41	38*
75	39	51	44	40	35	39
90	35	54	44	46*	35	38
105	31	44	32	54	31	36
120	28	40	24	50	29	35
135			13	42	23	32
150			8	6	20	29
165			6	1	15	29
180			3	0	13	27
195					6	21
210					2	19
225					1	15

Asterisks indicate time of addition from side arm. Trypanosome substance 0·024 ml.

TABLE VII

Time in minutes	O_2 uptake in μl.	
	Rabbit	Horse
15	81·3	78·4
30	78·0	83·0
45	69·2	84·9
60	62·4	78·4
75	52·3	65·2
90	59·6	65·9
105	44·2	58·2

In the case of trypanosomes, therefore, it would appear that it is the formation of acid, with consequent lowering of the pH, which represents the ' harmful products ' produced in the medium, to which some authors refer.

The amount of acid produced in terms of monobasic acid was determined by titration, addition of 0·1 NaOH being made to known quantities of mixture

after shaking in presence of an indicator (phenol red), until the tint matched that before shaking. More accurate determinations were made by determining the pH electrometrically (by glass electrode), both before and after shaking, and noting the amount of added NaOH required to give the observed shift in pH.

In these determinations, it was necessary also to take into account changes in the serum present as a result of the shaking at 37° C. This was most conveniently done by titrating also the mixture in the left-hand flasks, which usually showed some increase in pH due to removal of CO_2, the amount of 0·1 HCl required to bring to the original pH being added as a correction to the previous figure obtained. The increased alkalinity in the control (serum) flasks (pyrex) was at first thought to be due to creep of NaOH from the cups, but experiment showed that this was not so, and it appears certain that the explanation given above is the correct one, and that the serum maintained at 37° in presence of potash loses CO_2 and thereby shows an increased alkalinity. The same change must occur in the experimental as in the control flask, and therefore the correction noted has to be applied in arriving at the total acid produced by the trypanosomes. The results of these determinations are given in the next section.

Relation of O_2 Uptake to Glucose Consumption and Acid Production

The relation of O_2 uptake to glucose consumption in our experiments where sugar estimations were made, is given in tabular form below. A standard amount of parasite substance was used in each case; the duration of the experiment was two hours.

Table VIII

Normal or arsenic-fast	Drug used	μl. O_2 used	Mols O_2 $\times 10^6$	Per cent. of glucose before	Per cent. of glucose after	Per cent. of glucose used up	Mols glucose $\times 10^6$	Glucose/ O_2
Normal	Nil	474	21·3	0·231	0·103	0·128	21·3	1·0
Arsenic	,,	540	24·2	0·235	0·090	0·145	24·2	1·0
Normal	,,	491	22·0	0·231	0·070	0·161	26·8	1·2
,,	Quinine 0·005	33	1·48	0·231	0·222	0·009	1·5	1·0
Arsenic	R.T.* 0·00001	540	24·2	0·235	0·089	0·146	24·3	1·0
,,	R.T.* 0·0001	234	10·5	0·235	0·170	0·065	10·8	1·0
Normal	C.1† 0·000001	151	6·8	0·231	0·163	0·068	11·3	1·7

* Reduced tryparsamide. † Camphor-10-arsinic acid.

Those estimations giving results for trypanosomes in absence of drug appear to give clearly a relation of glucose used to O_2 uptake of unity. Actually some allowance must be made for the 15 minutes during which equilibration in the Barcroft has been brought about. As this would be approximately 1/8 of the total, the figure of 1·0 would become of the order of glucose/O_2 = 0·9, or a little in excess of a molecule of O_2 is used per molecule of glucose.

Even in the case of inhibition by drugs, this relation appears in some cases to hold good.

From the results given in Table IX it is seen that under aerobic conditions, allowing for acid formed during equilibration in the first four experiments only, approximately 2 molecules of monobasic acid were produced for each molecule of oxygen used. The ratio of glucose used to acid produced is therefore approximately 1 to 2.

TABLE IX

The relation of O_2 uptake to (monobasic) acid produced

Normal or arsenic-fast	Duration of experiment in hours	μl. O_2 used	Mols O_2 $\times 10^6$	ml. NaOH added *	ml. HCl added †	Total acid 0·1 M formed in ml.	Mols acid formed $\times 10^6$	Acid/O_2 uncorrected	Acid/O_2 corrected for equilibration period
Arsenic	2	500	22·4	0·40	0·214	0·64	48·0	2·1	1·9
,,	2	546	24·5	0·60	0·22	0·82	61·5	2·5	2·2
Normal	2	471	21·1	0·55	0·20	0·75	56·2	2·7	2·4
,,	2	491	22·0	0·64	0·16	0·80	60·0	2·7	2·4
,,‡	1	331	14·8	0·30	0·02	0·32	24·0	1·6	
,,‡	2	528	23·6	0·46	0·10	0·56	42·0	1·8	

* ml. of 0·1 NaOH added to give original pH on right side with 4 ml. of mixture.
† ml. of 0·1 HCl added to give original pH on left side with 4 ml. of control mixture.
‡ Electrometrically determined.

In the last two cases pH readings were as given below :

	pH values	
	Right	Left
At equilibrum	7·12	7·6
After 1 hour	6·55	7·64
After 2 hours	6·10	7·77

Respiratory Quotient

For determination of the respiratory quotient, three pairs of manometers were used, the oxygen uptake of a suspension, which was the same in all six flasks, being measured in the usual way in the first pair. The second pair had $\frac{1}{2}$ ml. N/H_2SO_4 in the side arm, which was added at equilibration immediately after closing the taps, KOH being absent from the cups. Acid alone was present on the left side, and the CO_2 liberated from serum was thus obtained at equilibration point. In the third pair, filled in the same way as the second pair, the $\frac{1}{2}$ ml. acid was added at the end of one hour—the period for which oxygen uptake was measured—and gives the amount of CO_2 formed from parasites plus serum, less the oxygen used. The following is a typical calculation for normal trypanosomes :

O_2 uptake in 1 hour	335·3 μl.
O_2 uptake + CO_2 total output	115·5
CO_2 from trypanosomes + serum	219·8
CO_2 from serum alone	164·6
CO_2 produced by trypanosomes	55·2

$$\text{Respiratory quotient} = \frac{CO_2}{O_2} = \frac{55{\cdot}2}{335{\cdot}3} = 0{\cdot}16$$

Mean of normal trypanosome values = 0·155
,, arsenic-fast trypanosome values = 0·145

As in the case of oxygen uptake, the normal and arsenic-fast trypanosomes give very similar results.

Reiner *et al.* (1936) noted that very small amounts of CO_2 are given off under aerobic conditions, and none under anaerobic conditions by *T. equiperdum*. For *T. equiperdum* the value of the respiratory quotient found by them was 0·062, but for *T. lewisi* the value approximated to unity.

Nature of Respiratory Mechanism

From what is known of the nature of respiratory enzymes in organisms and tissues, it is probable that the oxidation of glucose with acid formation observed is brought about by a dehydrogenase. In favour of this is the fact that living suspensions of trypanosomes rapidly decolorize methylene blue when put up under appropriate conditions in Thunberg's tubes. We have also confirmed the fact (see accompanying paper), as noted by Fenyvessy and Reiner (1924), that KCN does not inhibit the respiration of trypanosomes.

It may also be accepted as probable that some substance exists, or is formed, capable of extending the process by combining with molecular oxygen (with or without the intervention of an enzyme), so that the observed oxygen uptake may thus be brought about.

In none of our observations as yet, however, have we been able to obtain evidence of the action of enzymes apart from the presence of intact organisms exhibiting characteristic motility. Experiments with lysing through the action of water, and by freezing and thawing, gave a negative result, in that when motility had been abolished there was no O_2 uptake. A somewhat similar relationship was constantly seen in our drug inhibition experiments, for, as noted in the accompanying paper, it was very difficult to establish by counts a difference between actual lethal effect and inhibition of O_2 uptake.

The following experiment very clearly shows the close relationship between O_2 uptake and maintenance of the organism in the intact (and motile) state. It has been noted previously in this paper that, when all glucose present in the medium is exhausted, O_2 uptake ceases and, further, the trypanosomes become sluggish, motionless, deformed or undergo lysis. It was suggested to us by Professor Topley that, on analogy with bacterial organisms, this would offer favourable conditions for demonstrating enzyme action where the organisms themselves were no longer intact, in that the only change introduced was deprivation of the substrate. An experiment was designed, therefore, as set out in Table X, by which glucose in the side arm was added at various intervals following exhaustion of this substance in the medium. It is clear from the results obtained that, though addition of glucose was effective in restoring O_2 uptake when this had been much reduced or even practically abolished, the time during which such effect could be produced was very short, not amounting to more than 15 minutes.

TABLE X

Giving O_2 uptake for respective 15-minute intervals of preparations originally containing 0·024 ml. trypanosome substance in serum–Ringer–buffer without added glucose. Glucose–Ringer–buffer to give 0·2 glucose added at successive 15-minute intervals after cessation of O_2 uptake (evidenced in all tubes after the 30-minute reading)

Minutes	μl. O_2				
15	41·1	48·6	52·4	48·7	46·6
30	19·8	22·2	23·8	25·5	16·9
45	4·1	5·6	6·4	5·4	5·6
60	22·0	0·5	1·8	0·3	0·3
75	48·4	−5·6	−0·6	0	−0·8
90	42·3	18·4	−5·8	−1·5	0
105	40·3	14·2	4·6	−2·7	−2·0
120	34·2	15·6	4·4	3·9	−1·7
135	32·4	10·9	0·6	4·5	3·1
150	31·6	13·9	0·6	1·7	−0·8
165	22·3	12·3	3·5	−2·4	0·8
Trypanosome count per μl. $\times 10^{-3}$ at end of experiment	29	16	3	0	0

Addition of glucose from side arm indicated by line.

The counts given indicate that, when motile organisms were no longer present, addition of substrate was entirely ineffective in restarting O_2 uptake. From these results we conclude that either enzyme action depends on the intactness of some structural arrangement present in the living organism, or the interaction of a chain of enzymes with coenzymes effective during life is in some way interfered with by ' death ' of the organism. It is possible that the rapid lysis seen in the case of trypanosomes when these organisms cease to be motile may be responsible for the close relation between enzyme action and motility, cessation of motility, lysis and cessation of respiration being thereby made almost coincident phenomena in point of time. In the case of trypanosomes, therefore, a distinction between lethal effect and inhibition of respiration becomes difficult, if not impossible, as is noted in the following paper dealing with inhibitive effect by drugs.

Summary of Observations on Trypanosomes

The most striking feature of trypanosome metabolism under the conditions noted is the utilization of glucose, the formation of acid products and the large oxygen uptake. There appears to be no difference in the nature or degree of these processes between the normal and arsenic-fast strains.

Deprivation of glucose is followed at once by cessation of O_2 uptake, and the normal content of glucose in a mixture of one part rabbit serum to three of total fluid is insufficient for full continued activity of these organisms beyond about 15–30 minutes at 37°.

The reduction in pH due to acid formation appears to be the cause, even in presence of glucose, of a gradual reduction in O_2 uptake, causing an increasing departure from the linear graph of such uptake. This fall-off in uptake is corrected by addition of alkali (buffer), and is less when horse serum, with stronger buffering action than rabbit serum, is used.

A mixture at 37° C. containing 54,000 trypanosomes per μl., suspended in rabbit serum, Ringer and buffer with 0·2 per cent. added glucose, showed initially a mean O_2 uptake for normal strain (53 observations) of 19·4 ml. O_2 per 10^{10} trypanosomes per hour, and for the arsenic strain (27 observations) one of 18·6 ml. per 10^{10} trypanosomes per hour. In terms of dry weight, the value was 285 ml. O_2 per gm. per hour (arsenic strain 275 ml. per gm. per hour). $Q_{O_2} = 285$.

This rate was not significantly different with pure (heated and unheated) serum, or horse serum in place of rabbit serum, or relatively within limits with differing quantities of material in the suspension.

The ratio of glucose used, and acid produced, to O_2 uptake was respectively about 1 and 2.

The respiratory quotient was very small, under 0·2, in both normal and arsenic-fast strains.

Deprived of glucose, trypanosomes rapidly become motionless, deformed and lysed. No enzyme bringing about O_2 uptake could be demonstrated from material lysed by water, or by freezing and thawing. Addition of glucose to organisms deprived of this substance for more than 15–30 minutes did not lead to renewal of O_2 uptake by the suspension. Cessation of movement, lysis, and disappearance of enzyme action, as evidenced by O_2 uptake, are in trypanosomes closely related, and are brought about as rapidly and effectively by deprivation of glucose as by addition of lethal drugs.

We have confirmed the fact, as noted by Fenyvessy and Reiner (1924), that KCN has no inhibitory action on respiration.

OBSERVATIONS ON *PLASMODIUM KNOWLESI*

So far as we are aware, no observations have been recorded on the respiration of malaria parasites.

Material and Methods

In all except special cases the 3·0 ml. of mixture in the right flasks contained 1·0 ml. standard suspension of parasite substance in monkey serum (usually obtained from the monkey from which the parasite substance had been obtained), 1·0 ml. Ringer solution and 1·0 ml. m/15 phosphate buffer pH 7·4. No glucose was normally added (see section on relation to glucose). The control-flask mixture was identical, except that serum was used in place of suspension. Two strengths of suspension were commonly used, namely, one of parasite substance in three of serum (1/4), giving in the flask 0·25 ml. of parasite substance, and a weaker suspension of one to nine of serum (1/10), giving in the flask 0·1 ml. parasite substance.

Parasite substance* was prepared as follows. Monkeys freshly inoculated were kept under observation until the parasites in the primary attack had increased so greatly that death was threatened. At the height of infection, and with 25 per cent. or more of the red cells infected by the large forms of the parasite, as observed in a blood film from a vein, the animal is anaesthetized with chloroform and cardiac puncture is performed. After all the blood possible has been withdrawn by this method, the chest is opened and a further supply obtained by direct puncture of the heart, aided by massage of the body and limbs. The total blood so obtained, usually from 60 to 80 ml., is defibrinated by gently shaking in a flask containing some thin glass rods.

The defibrinated blood, after filtering through gauze (or loose wool), is then centrifuged at 2,000 r.p.m. for 45 minutes, whereby the large forms of the parasite separate as a brown layer above the red cells. This layer consists

* Although such material has not been used by us in the results given, a further considerable supply of parasite material can be obtained after bleeding by tying a canula into the ascending aorta, perfusing with a solution of 2 per cent. citrate containing 0·45 per cent. saline, and collecting the returning fluid by means of a canula tied in the right auricle.

almost entirely of parasites from half to three-quarters grown or more, surrounded by the remains of the parasitized red cells. This brown layer is pipetted off and recentrifuged for 45 minutes at the same speed in graduated tubes of small bore containing about 2 ml. of material. The considerable volume of brown material so obtained, sharply differentiated from the red cells, can then be removed in required measured amounts. Such deposit is referred to by us as ' parasite substance.' One volume of parasite substance suspended in three volumes of the infected animal's serum, or one volume in nine volumes of serum, according to circumstances, constitutes our 1/4 and 1/10 standard suspensions. Though ' parasite substance ' so prepared may contain also a few leucocytes and a few uninfected cells, the volume of these is relatively very small compared with the mass of parasitized red cells, and, though some smaller parasites may be present, the larger forms greatly preponderate. The following are typical percentage differential counts of suspensions :

	1	2
Full-grown schizonts	12·4	6·7
Three-quarters-grown schizonts ..	76·1	86·6
Half-grown schizonts	6·7	1·7
Rings and small forms	0·4	0·2
Leucocytes	0·2	0·2
Uninfected red cells	4·2	4·6

This relative uniformity of the product tends to be maintained by the method of preparation, for not only is the period of maximum development of the parasites chosen when killing the animal, but only cells containing parasites sufficiently large to render them a certain degree lighter tend to be brought to the top by centrifuging. By killing the animal at not too late a stage, the presence of leucocytes can be almost entirely avoided.

Following the work of Sinton and Mulligan (1933), we have, by splenectomizing chronically infected and immune stock animals, been able to obtain infections similar to the primary infections from which our material has been prepared. Not only so, but the yield of material obtained has been extremely large, possibly the result of decreased phagocytosis.

In the preparation of parasite substance, a very striking feature is the rapidity with which blood containing numerous parasites darkens, becoming chocolate-coloured or even almost black. The change is very markedly shown in the case of centrifuged red cell deposit containing parasites when allowed to stand a short time. Nauss and Yorke (1911) observed a darkening of the red cells when trypanosomes were present, due to the taking up of oxygen by these parasites. The blackening of the blood containing numerous parasites of *P. knowlesi* is, however, far greater than we have ever seen, even with the most

heavily infected trypanosome blood. On shaking such blood or deposit, it largely regains its bright-red colour, but rapidly darkens again.

Inoculation of animals with mixtures containing parasite substance after two hours' shaking in the Barcroft manometer gives rise to infection as with fresh blood, but what proportion of parasites are living under various circumstances has not yet been determined by us, as such determination offers considerable difficulty. Attempts to differentiate ' living ' from ' dead ' parasites by intravitam staining methods have not as yet been successful. Microscopical examination of material after shaking in the Barcroft apparatus has not shown any marked changes from appearances seen in fresh material. Frequently, however, the amoeboid-like shape of the parasites is very marked.

The parasite content of the standard mixture made from suspension 1/10 was found, as a mean of a number of determinations, to be 246,000 per μl. The number of parasites present in the 3·0 ml. of mixture used in the flask of each Barcroft manometer was, therefore, $7{\cdot}38 \times 10^8$.

The weight of 1 ml. of compacted red cells (centrifuged for 45 minutes at 2,000 r.p.m.) was found, as the mean of three observations, to be 1·086 gm., and the dry weight 0·270 gm. The mean weight of 1 ml. parasite substance, given as the mean of three observations, was 1·061 gm., and the dry weight was 0·190 gm.

On account of the presence of attached red cell substance, determination of the actual amount of parasite present in the mixture offers some difficulties. The wet weight of 1 ml. of parasite, as prepared by washing parasite substance in hypotonic salt free from haemoglobin and a final wash in 0·9 salt, was 1·049 gm., and the dry weight was 0·041 gm. Since the volume of red cell substance in parasite substance estimated from the haemoglobin washed out is normally about 25 per cent. of the volume of parasite substance, the dry weight of parasite in 1 ml. parasite substance should be approximately 0·03 gm., and the amount of actual parasite in dry weight in each flask 0·003 gm. when using 0·1 suspension, and 0·0075 when the 0·25 suspension was used. Actually it is not known what loss results from washing in hypotonic salt, so that the estimate may be too low.

O_2 Uptake by Malaria Parasite Substance

The following mean uptakes in μl. for the successive 15-minute intervals were obtained using 0·25 ml. of freshly prepared parasite substance, in the absence of added glucose. The means are arranged in classes on the basis of one hour's uptake.

TABLE XI

15-minute intervals	O_2 uptake in μl. per hour				Total no. of observations	Mean experimental uptake	Mean uptake per ml.
	200–	250–	300–	350–			
1	60	73	86	101	11	84	336
2	58	71	83	103	11	83	332
3	54	66	83	91	11	78	312
4	51	66	80	88	11	76	304
5	51	60	75	77	9	70	280
6	46	56	72	73	9	66	264
7	45	55	67		4	59	236
8	44	55	66		3	56	224
No. of experiments	2	1	5	3			

Uptake per ml. during first hour, 1,284 μl. O_2.
" " " second " 1,026 μl. O_2.

Four observations with the same material kept overnight in the ice-chest gave a mean uptake per ml. per hour in the first hour of 928 μl., and in the second hour of 760 μl.

With 0·1 ml. of fresh substance the results were as follows :

TABLE XII

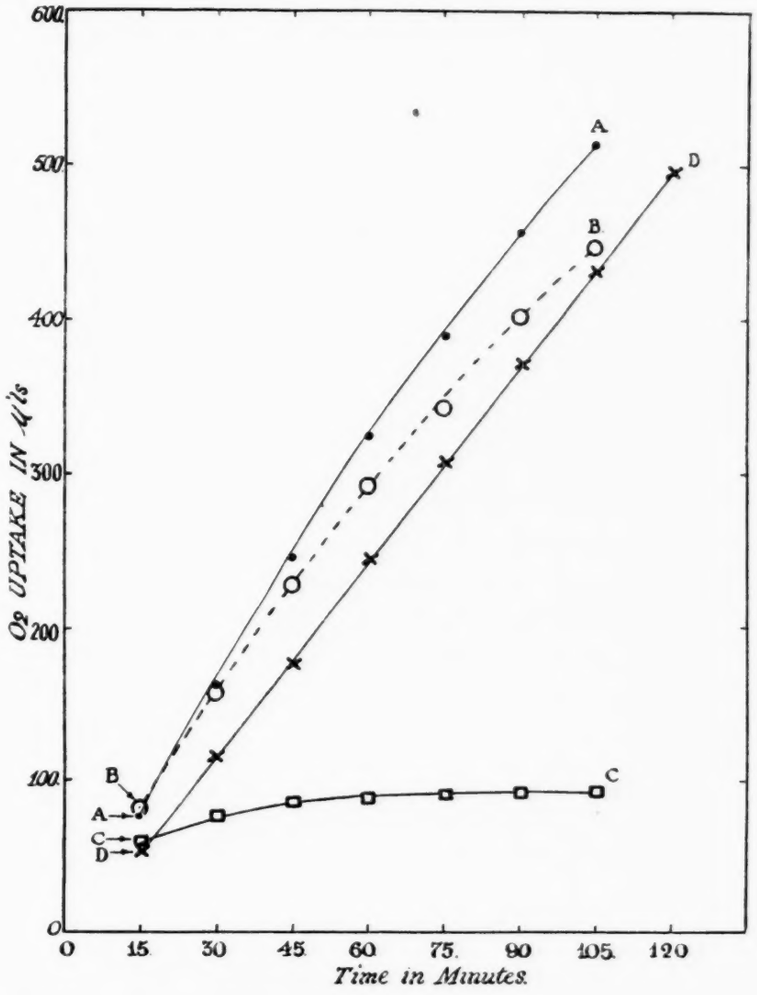
15-minute intervals	O_2 uptake in μl. per hour			Total no. of observations	Mean experimental uptake	Mean uptake per ml.
	100–	150–	200–			
1	32	44	55	16	39	390
2	32	46	62	16	41	410
3	32	44	59	16	40	400
4	31	43	59	16	39	390
No. of experiments	7	8	1			

Uptake per ml. in the hour, 1,590 μl. O_2.

Four observations on the same material kept overnight in the ice-chest gave a mean uptake per ml. per hour of 928 μl. O_2.

These results, showing continuous uptake for two hours in the absence of added glucose, are in marked contrast to the findings for trypanosome suspensions under the same conditions. The gradual falling off in O_2 uptake with time is also clearly less than in the case of trypanosomes, even when these have been provided with glucose. With 0·1 suspension, there is commonly no fall-off within the two hours, and preparations run for 10 hours have in some cases shown only a very gradual reduction in the rate of uptake. The linear nature of the uptake is well shown in the characteristic graph, which also

demonstrates the higher buffering power of horse serum with trypanosomes. The contrast with trypanosomes in this respect is therefore also very marked. In addition, material kept 24 or even 48 hours in the ice-chest usually gives a considerable, although reduced, uptake, whereas trypanosomes cease to be capable of giving any uptake, even in the presence of sugar, if deprived of this substance for half an hour.



O_2 uptake curves for malaria parasites and trypanosomes.

A. Standard trypanosome suspension in horse serum + glucose.
B. Similar „ „ rabbit „ + „
C. Same „ „ „ „ with no glucose added.
D. 0·1 malaria parasite suspension without added glucose.

Though it has been noted that the deposit of parasite substance tends to be roughly similar in character as prepared on different occasions, still a certain variation occurs. Yet, in spite of the possible variations in the material used, the results are surprisingly constant and suggest that uptake is proportional to amount of parasite material. The somewhat higher relative uptake with 0·1 suspension over that with 0·25 may be due to more suitable conditions for

respiration with the weaker suspension. The relation of amount of O_2 uptake to quantity of material is also shown in the series given below, which, however, is not comparable with the previous data, since m/3 buffer was used in making up the mixtures. The figures in all cases are reduced to μl. per ml. of parasite substance per hour.

TABLE XIII

Time in minutes	Ml. parasite substance used			
	0·25	0·20	0·125	0·10
15	704		704	680
30	656	770	640	720
45	576		576	760
60	480	490	320	680
75	528			600
90	432	320	256	560
105	352			520
120	256	260	128	400

Taking, as before, observations for the first half hour and the figures for O_2 uptake given by 1/10 suspension, the O_2 uptake per 10^{10} parasites per hour is 2·17 ml., or per gm. dry weight actual parasite 53 ml. ($Qo_2 = 53$). As compared with the corresponding values for trypanosomes, the uptake per gm. dry weight, according to the estimate, is some 5–6 times smaller. This figure might require to be somewhat increased—say, to possibly 8–10 times smaller—if the estimated weight of parasite be considered as probably too low. As compared with the same number of trypanosomes, the uptake is some 9 times smaller. Per ml. of parasite substance (i.e., the unwashed material) uptake per hour is 1·60 ml., as against 8·72 ml. for trypanosome deposit, or again about 5 times less.

In addition to using parasite substance, we have also carried out some observations using the whole blood from heavily infected monkeys. In these cases, the uptake was much greater than that with normal blood and roughly proportional to the parasite content. The uptake was sufficiently large in such heavily infected blood to make its use quite practicable in the measurement of O_2 uptake, though as yet we have not systematically followed up this line of enquiry.

THE EFFECT OF VARIOUS SUBSTRATES ON O_2 UPTAKE BY MALARIA PARASITES

Glucose. The most noticeable feature of O_2 uptake with malaria parasite substance as compared with trypanosomes is that uptake proceeds independently of any added glucose, and that there is no abrupt falling off at any time, as is so

markedly shown by trypanosomes, indicating exhaustion of sugar in the mixture. We have carried out experiments in the Barcroft apparatus for as long as 10 hours, and, while uptake became progressively less, its fall-off was gradual and in no way corresponds to the abrupt change seen with trypanosomes when glucose in the medium is exhausted. Addition of glucose to mixtures has shown no distinct effect upon O_2 uptake. In some cases, the readings have been slightly greater, in others a slight decrease was seen, but in no case were the differences large.

Although the above considerations appear to show that glucose is not necessary for oxygen uptake, nevertheless, determination of the sugar content of mixtures has shown that with or without addition of glucose up to 0·2 per cent. there is a rapid disappearance of sugar within half an hour, even at ordinary temperatures and without shaking in the Barcroft. Since red blood corpuscles are known to cause glycolysis, controls were set up with normal blood cells and serum in place of parasite substance, but practically no loss of sugar was found over a period similar to that in which the parasite substance under experimental conditions caused complete or almost complete disappearance of sugar.

In an experiment with 0·2 per cent. glucose added above what is present in serum and 717 million parasites, we found that the amount of glucose used up was as follows :

1 hour	1·62 mgm.
2 hours	3·24 ,,
7 ,,	4·68 ,,

In this case there was a 23 per cent. increase in oxygen uptake over that in the controls with no added glucose. Generally the difference in O_2 uptake has not amounted to more than 3 or 4 per cent. In another experiment, with 2,889 million parasites, where the original mixtures contained 175 and 355 mgm. glucose per 100 ml. respectively, there was no difference in O_2 uptake ; 4·74 mgm. glucose was used up in the former case and 4·47 in the latter. In this experiment the fall-off in O_2 uptake was rather abrupt after opening and reclosing of the taps. Whether the high glucose content was harmful in the long run we cannot say.

In a further experiment with whole blood, in which 1/10 of the cells were infected by large forms of the parasite, 679 million parasites in 3 hours at 37° in sterile flasks appeared to use up 4·80 mgm. glucose. With the same blood under culture conditions for 9½ hours with passage of oxygen, 7·8 mgm. of glucose were used. In a control preparation kept in the ice-chest only 0·6 mgm. glucose disappeared. Another point of interest is that sugar content of the mixture, whether with or without 0·2 per cent. final added glucose, falls to the same value within an hour or so, without any noticeable effect on O_2 uptake.

The fact that in the majority of cases added glucose has not caused an increase in O_2 uptake may, it was thought, be due to the fact that the parasites carry an oxidizable substrate with them. The glucose content of the actual parasite substance was therefore estimated in a number of cases by the Hagedorn-Jensen method and found to be about 20 mgm. per 100 ml., a figure far below that of the serum or whole blood.

In view of our findings with trypanosome-infected mice, the possibility arose that similar experiments with monkeys infected by malaria parasites might throw some light on the relation of blood sugar to parasite numbers, and the sugar content of the blood of infected animals has been regularly determined. The relation between the number of parasites in the blood and the value of the blood sugar is not constant, owing in part probably to the excitable nature of the animal. But monkeys with heavy infections have definitely a lower blood sugar. The following results were obtained for some 20 animals. The blood sugar for a normal animal was found to range from 90 to 120 mgm. per 100 ml.

Infection	Average mgm. glucose per 100 ml.
Small	101
Medium	110 (2 values being very high)
Heavy	78

The reduction noted even with the enormous infections seen in *P. knowlesi* is, however, not comparable with that seen in trypanosome-infected mice, and other causes of reduction in blood sugar than direct using up by the parasites in such cases is quite possible.

A further point relevant to the present considerations is the generally accepted view that addition of glucose to blood is necessary for successful cultivation of malaria parasites. Following the paper by Bass (1911), in which he states that he had cultivated *P. vivax*, *P. malariae* and *P. falciparum* (without giving full details of technique), Sinton (1912) failed to corroborate his results, as he had not added glucose. Thomson and McLellan (1912), following a second paper by Bass (1912) and another by Bass and Johns (1912), were able to cultivate one generation of *P. falciparum* by Bass's method, when it was made known that dextrose must be added to the culture medium. In the paper by Bass and Johns, the statement is made that ' dextrose is always required,' and also that it may be replaced by maltose but not by other sugars tried. They are of the opinion that dextrose, besides serving as a nutritient medium, may also play some physico-chemical rôle. It may be pointed out that addition of glucose to the blood can through glycolysis have the effect of lowering the pH of the serum, which, as in our Barcroft experiments, otherwise shows a raised pH value due to loss of CO_2.

Though there is evidence that addition of glucose favours or may even be essential to culture of malaria parasites, and though we have found a remarkable disappearance of glucose in mixtures containing parasites, we do not think that O_2 uptake by malaria parasite substance can be dependent on glucose consumption, as in the case of trypanosomes. Since parasite substance per unit of dry weight shows a much smaller O_2 uptake than that shown by trypanosomes, it might be held that there is sufficient glucose present in the blood or even in the red cell, without the necessity of any addition, to enable the parasites to continue their respiration without exhaustion of glucose in the time given. In our experiments, however, the amount of parasite substance (dry weight) used is much greater than that of trypanosomes, and the total O_2 uptake in the flask is at least equal to that of trypanosomes. With this total uptake, glucose should, therefore, be equally rapidly used, whether present in the serum or in the red cell, and, as in trypanosome experiments, fall-off in uptake should rapidly occur in the absence of added sugar. Further, as shown later, the formation of acid, so characteristic of trypanosome metabolism, is not seen with malaria parasites, indicating that, even if glucose were utilized, the mechanism of O_2 uptake is not the same in the case of malaria parasites as in trypanosomes.

Glycogen. In order to find if the disappearance of glucose was due to storage by the parasite in the form of glycogen, several estimations of the glycogen content of the parasite substance were made. The glycogen was determined by Pflüger's method in the usual way.

Experiment 1. Glycogen absent in parasite substance 2 days old.
" 2. Fresh parasite substance 0·17 per cent.
" 3. { " " 0·036 " } { " " left overnight 0·022 per cent. }
" 4. " " 0·057 per cent.
" 5. " " 0·047 "

The part played by glucose in the metabolism of the malaria parasite requires further investigation, especially in relation to certain features of our experiments discussed later.

Succinate, lactate and glutamate. Succinate 0·005–0·02 molar concentration has in some cases given rise to an increase in O_2 uptake over the control (in one case amounting to 23 per cent.), but it seems doubtful if the results are significant, as in other cases no increase, or even a decrease, has been observed. Neither lactate nor glutamate appeared to have any significant effect.

Haemoglobin. Various facts have led to the belief that the haemoglobin of the parasitized red cell is utilized by the growing parasite. Some of these, such as the pallor of the affected cell especially seen in benign tertian, and the presence of haemoglobin derivatives in the serum and tissues, are not certain proof of this, since haemoglobin may merely be liberated. The formation of pigment in the parasite, which has been proved by Sinton and Ghosh (1934)

to be haematin, appears, however, to remove any doubt on this point. Nevertheless, when investigating the metabolism of the malaria parasite it seemed important to determine experimentally whether, in preparations showing oxygen uptake, there was a decrease in haemoglobin. Since the total life-cycle of *P. knowlesi* is one of 24 hours, during which period the whole of the haemoglobin of the infected cell is seemingly used up, it is to be expected in parasite substance, where only parasite-containing red cells are present, that a considerable reduction in the total haemoglobin of the preparation would be demonstrated after several hours' metabolism.

Attempts to show a reduction in amount of haemoglobin in parasite substance mixtures, and in heavily infected blood by colorimetric methods, on the basis of the Sahli technique, failed to give significant differences after 2–10 hours' shaking. More delicate observations were therefore carried out, based on measurements of haemoglobin by O_2 capacity, the material used being freshly defibrinated, heavily parasitized blood. The method used, which by preliminary trial was found to give consistent results, was as follows. To the right-hand flasks of two (or more) Barcroft manometers containing KOH in the cups, are added 2 ml. blood accurately measured, the last drop expelled by a teat. This was followed by twice the volume of dilute ammonia (prepared from 4 ml. ammonia sp. gr. 0·880 diluted to 1 litre with distilled water) and the flasks were equilibrated for 15 minutes. After equilibrition 0·5 ml. of fresh saturated ferricyanide was added from the side arm, and readings were taken after 5 and 15 minutes' further shaking. In the left flask ferricyanide was replaced by Ringer solution or saline, the contents being otherwise similar. Complete oxygenation of the blood before measuring was ensured by rotating in a stream of oxygen for 2 minutes, followed by shaking 15 minutes in air. In all cases the values obtained for the different manometers corresponded closely. The following table gives the results obtained :

TABLE XIV

O_2 capacity of 2 ml. infected blood in μl.

	A		B	
	5 minutes	15 minutes	5 minutes	15 minutes
Original blood	123·1	111·8	132·1	121·4
Shaken 9 hours at 37° in sterile flasks	78·6	73·7	114·6	108·3
9 hours' culture at rest	114·6	109·6		
9 hours' culture with O_2 passing ...	104·6	104·2	130·6	125·2
3 hours shaken			127·4	118·1

The table gives the O_2 capacity equilibrated for 5 minutes and 15 minutes after addition of ferricyanide, as we noted a continual fall in oxygen liberated,

as if it were being reabsorbed. When estimations were made with normal blood shaken for 9 hours, the values remained constant. There was also no loss of haemoglobin. We concluded that there is a definite loss of Hb. in parasitized blood, more in the samples shaken at 37° in the Barcroft apparatus than under culture conditions, and that the loss of Hb. increases with time of shaking. The oxygen capacity as given is directly proportional to the amount of Hb. present. Absolute and differential counts for A and B were respectively :

	A	B
Absolute count per μl.		
Parasites	827,000	235,000
Uninfected red cells	2,065,000	2,147,000
Differential count of parasites		
Full grown schizonts	12·2	2·8
Three-quarters-grown schizonts	57·6	52·4
Half-grown schizonts	18·0	32·0
Smaller forms	12·2	12·8

The oxygen uptake as observed in two manometers with 3·0 ml. parasitized blood was as follows :

O_2 uptake by 3 ml. blood in μl.

Time in minutes	60	90
A	171·4	232·8
B	110·2	153·7

It will be noted that the loss of Hb. is distinctly greater in experiment A than in B ; in the former case a much larger proportion of parasites to the total cells is present, and provides some additional evidence to that obtained with normal blood for the belief that loss of Hb. is due to the presence of parasites. The oxygen uptake is, moreover, also considerably greater in A than in B. The values for O_2 uptake and haemoglobin used up, assuming that the experiments indicate some form of alteration of the oxyhaemoglobin, cannot, it is obvious, be accurately compared.

Changes Produced in the Medium

Acid production

In order to ascertain the acid production, if any, by parasite substance, pH determinations by the glass electrode have been made, both of the supernatant fluid and of the laked deposit from the standard mixture, before and after shaking in the Barcroft apparatus. For the observations on the laked deposit,

the material (parasite substance) obtained after the first centrifuging and removal of the supernatant fluid was made up to the original volume of the fluid with distilled water, mixed, and after a suitable interval again centrifuged, the pH of the supernatant fluid so obtained being measured.

Similarly, observations were made with red cell deposit from a heavily infected animal. In this case numerous small parasites were present.

Determinations of pH were made by the method described by one of us (Christophers, 1937), a specially devised small container being used, suitable for the small quantities of fluid available. By this means the pH can be determined for volumes of 2 ml. or upwards. The results obtained were as follows, the figures relating to determinations on two monkeys, 1 and 2, as indicated by the figures in brackets :

	pH before	pH after	Time shaken	O_2 uptake
Parasite substance				
Supernatant fluid...	7·28 (1)	7·29 (1)	2 hours	411 µl.
	7·52 (2)	7·78 (2)	4 „	541 „ *
Laked deposit	7·06 (1)	7·11 (1)	2 „	411 „
	7·09 (2)	7·33 (2)	4 „	541 „ *
Red cells heavily infected monkey				
Supernatant fluid...	7·28 (1)	7·22 (1)	10 „	223 „
Laked deposit	6·98 (1)	7·10 (1)	10 „	223 „

* Amount in 1½ hours, the preparation being run with taps open for a further period up to 4 hours.

These results give no indication of any acid formation during the period in which preparations are kept in the Barcroft at 37° C., and in which a large O_2 uptake has been maintained.

In coming to the above conclusion, it is necessary to give consideration to the fact that in this case, in contrast to trypanosome mixtures, the presence of red cell substance introduces the possibility of a powerful buffering action. With normal red cell deposit in suspension 1 ml. 0·02 HCl is required to be added to 1·0 ml. r.b.c. deposit, to give a shift of pH from 7·36 to 7·06. A considerable amount of acid in the presence of r.b.c. substance might, therefore, exist without giving rise to striking pH effects. In the results obtained, however, there was no indication of even a small reduction in pH during the period of shaking, and, moreover, in the parasite substance mixtures there is only a relatively small amount of red cell substance present (approximately ¼ of 0·25 ml.). If for the same O_2 uptake acid was produced on the same scale as with trypanosomes, it would be readily detectable.

What has been brought out rather strikingly is the low pH of the red cell material derived from laked parasite substance (pH 7·1 or less, as compared with

7·3 or over in the normal monkey). The same reduction has, however, been found in the red cells of heavily infected monkeys from which the larger parasites have been removed by centrifuging. At the stage at which the blood is taken, the animal is, however, markedly cyanotic, and the change in the cells may possibly be due to associated conditions.

Amino nitrogen

If, as is generally assumed, and as is supported by our results, haemoglobin is broken up owing to the presence of the parasite, it seemed probable that, if the globin part of the molecule were metabolized, changes in the value of the amino nitrogen content would be found. For the purpose of estimation, the Van Slyke apparatus was employed, with the following results :

Ml. of amino nitrogen in ml. fluid

	Original blood	Incubated at 37° for 4 hours	Shaken in Barcroft for 4 hours at 37°	O_2 uptake in ml. for 90 minutes
Heavily infected whole blood	1·55	1·56		
Heavily infected whole blood and 0·2 per cent. glucose		1·54		
Infected r.b.c. 1 to 4 serum	1·37	1·48		
Standard mixture parasite substance ...	0·48		0·57	541
Standard mixture parasite substance and 0·2 added glucose	0·48		0·51	586

The differences in amino nitrogen content are small and not significant, but indicate a slight increase in the case of blood shaken in the Barcroft apparatus, as with change in haemoglobin content in corresponding experiments. If proteolytic enzymes are present and give rise to the formation of amino acids from globin of the general type $R.CH.NH_2.COOH$, further change of these amino acids might result as (1) $R.CH.NH_2.COOH \rightarrow R.CH_2.NH_2 + CO_2$ (2) $RCH.NH_2.COOH \rightarrow NH_3 + R.CH_2.COOH$. In the case of (2) no change in amino nitrogen would be detectable, and our results with pH determination show that no acid is formed. Changes in NH_3 content are given below.

Change in Non-Protein Nitrogen during Metabolism

In order to investigate the change in non-protein nitrogen in the original infected blood, in similar specimens kept in the ice-chest or shaken in sterile Barcroft manometers, as well as in that kept under culture conditions for the same length of time, the following procedure was adopted.

One volume of blood, two volumes of water and one volume of trichloracetic acid were mixed, and after centrifuging till clear the supernatant fluid was removed. Duplicate specimens of 1·5 ml. of the supernatant fluid were combusted in Pregl's micro-Kjeldahl apparatus, and the total nitrogen was estimated. Two ml. of a mixture consisting of

100 ml. conc. H_2SO_4 (nitrogen free)
5 ml. of 5 per cent. $CuSO_4$ in water
300 ml. syrupy phosphoric acid

were used for combustion. In the last two experiments the ammonia content of 4 ml. of the fluid was estimated by making the latter alkaline with potassium carbonate and aerating into standard acid. These results are collected below.

N.P.N. and ammonia content of supernatant fluid recorded as ml. of N/70 HCl

	(1)	(2)		(3)	
	N.P.N.	N.P.N.	NH_3	N.P.N.	NH_3
Original blood	1·50	1·57		1·74	
	1·46	1·47	0·16	1·76	0·21
7 hours in ice-chest ...	1·51				
	1·52				
Shaken for 9 hours at 37° in Barcroft apparatus	2·05	2·31		2·96	
		2·11	0·15	2·92	0·19
9 hours' culture at 37°...	1·48	1·91		2·54	
		1·91	0·17	2·56	0·16
22 hours' culture at 37°		2·39			
		2·35			
Differential count of blood	500 parasites 4,762 non-infected r.b.c.	500 parasites 1,390 non-infected r.b.c.		500 parasites 895 non-infected r.b.c.	

It is clear from these results that there is an increase in the amount of non-protein nitrogen both in the blood shaken in the Barcroft apparatus and in that of the culture series. The differential counts given in the table show, moreover, that the change is greater where the parasites are most numerous relative to the number of red cells. As there is no change in the blood kept in the ice-chest, where metabolic processes presumably cease, it seems clear that protein is altered during metabolism of the parasites. The results given for ammonia indicate that this substance is not produced during metabolism. The amounts present are very small in all the samples.

Respiratory Quotient

This value was determined in the same manner as with trypanosomes, all six flasks containing suspension, so that the effect of any CO_2 formed during

equilibration, or in fact any changes occurring prior to closing of the taps, was eliminated. 0·2 per cent. glucose was added in certain cases. The following results were obtained, based on one hour's uptake.*

Amount of parasite substance	0·1	0·1	0·1	0·25
0·2 per cent. glucose	—	+	+	+
O_2	83·9	211·4	179·8	349·0
$O_2 + CO_2$ (total)...	−32·8	−69·0	−52·8	−60·4
CO_2 (total)	116·7	280·4	232·6	409·4
CO_2 other than from parasite metabolism after closing of taps	43·6	93·4	90·1	83·8
CO_2 output by parasites	73·1	187·0	142·5	325·6
Respiratory quotient CO_2/O_2	0·87	0·86	0·79	0·93

Mean respiratory quotient 0·86.

Nature of Respiratory Mechanism

Part played by serum and the red cell in O_2 uptake

In order to ascertain, if possible, to what extent serum in the mixture plays a part in O_2 uptake, some experiments were undertaken in which the amount of serum present was much reduced or absent.

In one experiment, in which parasite substance was washed once in isotonic saline and made up in mixture with Ringer and buffer only, the O_2 uptake was much reduced (control uptake 360 μl., uptake in absence of serum 44 μl.).

As it was thought that physical changes might have played a part other than absence of serum, an experiment was undertaken in which deposit was washed in Ringer and buffer and made up as above. The uptake was here again reduced considerably (control 502 μl., uptake in absence of serum 138 μl.). A very similar result was obtained with material washed once in citrate and put up without addition of serum in Ringer and buffer (control 556 μl., absence of serum 138 μl.).

An experiment designed to maintain the physical conditions as far as possible with a minimum of serum was carried out as follows. An amount of parasite substance to give 0·25 ml. deposit in each of three flasks was mixed with an equal quantity of red cell deposit in serum and centrifuged ; the serum as far as possible was removed, and the deposit made up with Ringer and buffer. The control-flasks mixture was prepared in an exactly similar way, but after centrifuging the

**Note.* Since the paper was sent to press we have carried out five further determinations of RQ for malaria parasite substance, giving, in absence of added glucose, values for RQ of 0.83, 0.98, 0.98, 0.97 and 1.00, the mean for the whole series, including those already given, being 0.91.

serum was not removed, the deposit being resuspended. O_2 uptake for two hours showed only a moderate falling off (mean of control flasks 518 μl., mean of flasks with trace of serum only 476 μl.).

It would appear that in the presence of even minimal amounts of serum O_2 uptake by the parasite under certain conditions may remain practically normal.

Effect of cyanide

Mixtures put up with 0·001 KCN showed complete inhibition of O_2 uptake during the first half hour, with subsequently a very small uptake, the uptake for two hours being : control 308 μl., and KCN flasks 26 μl. This effect suggests interference with O_2 uptake through combination of the cyanide with the parasitized red cell substance, or a direct effect upon enzymes of an oxydase type.

It is thought, as a result of experiments upon the inhibitive action of drugs, that those which affect the red cell in a marked degree, e.g., methylene blue, are particularly effective against parasite substance. This would suggest that the parasite is very vulnerable through the red cell, and it seems not improbable that oxygen is normally obtained through the oxyhaemoglobin of the cell.

Effect of narcotics

Enzymes of the dehydrogenase type are known to be inhibited by narcotics, e.g., urethane. The effect of urethane in different concentrations upon parasite substance and upon trypanosomes is recorded in the following paper. The inhibitive effect of these drugs is obtained in only relatively high concentration. Urethane, being an ester and non-ionized, can be safely used in such concentrations, without giving salt effect.

Unfortunately, in this case it is not merely an enzyme which is necessarily concerned, for the rather strong concentrations of urethane which it is usual to employ (5 per cent.) may well affect the parasites as such, and a lethal effect upon the parasite may, as has been seen in the case of trypanosomes, itself lead to cessation of respiratory enzymes. It would seem, therefore, that any conclusion as to the presence or absence of dehydrogenase systems cannot be drawn from these observations.

Summary of Observations on Parasite Substance

The most outstanding feature of O_2 uptake by parasite substance is its steady continuance, with little or no falling off, for very considerable periods, in the absence of added glucose and apparently independently of the normal presence of this substance, either in the serum or in the red cell.

That oxygen can be obtained from the oxyhaemoglobin of the cell is clearly shown by the remarkable change in colour when parasitized cells are excluded from contact with air. This change in colour, indicating reduction of oxyhaemoglobin, is far more rapid and marked than that seen with trypanosomes in blood,

as first observed by Nauss and Yorke (1911), and the process in the case of parasites must be one of great intensity.

Whether this action is dependent upon the presence of an oxydase, upon that of an auto-oxydizable substance stored in the parasite, or upon some form of oxidizable and reducible substance (hydrogen carrier), and whether in the latter case the system works through a concomitant dehydrogenase enzyme, we are as yet not in a position to say.

Our observations, however, show that in medium containing parasites (1) there is a very considerable consumption of O_2; (2) there is an approximately equal output of CO_2; (3) haemoglobin is altered, if not actually split up; (4) non-protein nitrogen is increased. Our observations also show (5) that there is no formation of acid comparable to that produced by trypanosomes; (6) that there is no detectable increase in NH_3. Further, we have shown that (7) serum appears to play but little part in providing the requirements of O_2 uptake; and that (8) the parasite can readily take oxygen from the oxyhaemoglobin of the cell.

REFERENCES

Bass, C. C. (1911). A new conception of immunity. *Jl. Amer. Med. Assoc.*, **57**, 1534.

——— (1912). Successful cultivation of malarial plasmodia. *Ibid.*, **59**, 936.

——— and Johns, F. M. (1912). The cultivation of malarial plasmodia (*Plasmodium vivax* and *Plasmodium falciparum*) *in vitro*. *Jl. Exper. Med.*, **16**, 567.

Biot, C., Biot, R., and Richard, G. (1911). Influence du glucose sur la vitalité du *Trypanosoma lewisi in vitro*. *C. R. Soc. Biol.*, **71**, 368.

Christophers, Sir S. R. (1937). Dissociation constants and solubilities of bases of anti-malarial compounds. I: Quinine; II: Atebrin. *Ann. Trop. Med. & Parasitol.*, **31**, 43.

Dixon, M. (1937). Respiratory carriers. (Needham and Green: Perspectives in Biochemistry. p. 123.) Camb. Univ. Press.

Fenyvessy, B. von (1926). Über die Bedeutung des Stoffwechsels der Parasiten für das Wirtstier bei der Trypanosomeninfektion. *Biochem. Ztschr.*, **173**, 289.

——— and Reiner, L. (1924). Untersuchungen über den respiratorischen Stoffwechsel der Trypanosomen. *Ztschr. f. Hyg. u. Infekt.*, **102**, 109.

——— ——— (1928). Atmung und Glykolyse der Trypanosomen. II. *Biochem. Ztschr.*, **202**, 75.

Jancsó, N. von, and Jancsó, H. von (1935). Chemotherapeutische Wirkung und Kohlehydratstoffwechsel: die Heilwirkung von Guanidinderivaten auf die Trypanosomeninfektion. *Ztschr. f. Immunitätsforsch.*, **86**, 1.

Kudicke, R., and Evers, E. (1924). Ueber den Einfluss von Zuckerarten und Alkoholen der Zuckerreihe auf die Beweglichkeit der Trypanosomen in vitro. *Ztschr. f. Hyg. u. Infekt.*, **101**, 317.

Lourie, E. M., and Yorke, W. (1937). Studies in chemotherapy. XVI: The trypanocidal action of synthalin. *Ann. Trop. Med. & Parasitol.*, **31**, 435.

Nauss, R. W., and Yorke, W. (1911). Reducing action of trypanosomes on haemoglobin. *Ibid.*, **5**, 199.

Reiner, L., Smythe, C. V., and Pedlow, J. T. (1936). On the glucose metabolism of trypanosomes. *Jl. Biol. Chem.*, **113**, 75.

Sinton, J. A. (1912). Some attempts at the cultivation of the malarial parasite by Bass's method. *Ann. Trop. Med. & Parasitol.*, **6**, 371.

——— and Ghosh, B. N. (1934). Studies of malarial pigment (haemozoin). pts. I, II, III. *Rec. Malar. Surv. India*, **4**, 15, 43, 205.

——— and Mulligan, H. W. (1933). Mixed infections in the malaria of the lower monkeys. pt. I: Mixed infections as the cause of apparent variations in the morphology and pathogenicity of Simian plasmodia. *Ibid.*, **3**, 719.

Thomson, J. G., and McLellan, S. W. (1912). The cultivation of one generation of malaria parasites (*Plasmodium falciparum*) *in vitro*, by Bass's method. *Ann. Trop. Med. & Parasitol.*, **6**, 449.

THE INHIBITIVE EFFECT OF DRUGS UPON OXYGEN UPTAKE BY TRYPANOSOMES *(TRYPANOSOMA RHODESIENSE)* AND MALARIA PARASITES *(PLASMODIUM KNOWLESI)*

BY

J. D. FULTON*

AND

SIR S. RICKARD CHRISTOPHERS†

(London School of Hygiene and Tropical Medicine)

(Received for publication January 27th, 1938)

In the preceding paper we have dealt with the normal respiratory metabolism of *T. rhodesiense* and *P. knowlesi.* The observations given in the present communication deal with the quantitative effect of known concentrations of various drugs and chemical compounds in reducing or entirely inhibiting O_2 uptake in the two types of parasites mentioned.

In the case of trypanosomes, Yorke and his co-workers (1929, 1935) have made important observations on the method of maintaining these parasites at 37° C. *in vitro*, and on the lethal effect upon them of various organic arsenical compounds. They have shown that *in vitro* the pentavalent derivatives exhibit a comparatively slight lethal effect, as compared with the powerful trypanocidal properties of many trivalent arsenic derivatives. The important question of drug-resistance has also been dealt with by them, through studying the lethal effect of different arsenicals on normal and resistant strains. Trypanosomes, made resistant to one organic compound of arsenic, exhibit the phenomenon in various degrees with all related compounds and with acriflavine. Ehrlich (1908) believed that resistance was due to some alteration in the nature of the chemoreceptors in the parasite. Voegtlin, Dyer and Leonard (1923) discuss the rôle of the sulphydryl group in the parasite, and consider that combination with the drug interferes with cell respiration. The point is of special interest to us. Resistance on this view demands an excess of sulphydryl groups. Voegtlin, Dyer and Miller (1924) suggest lessened permeability of the parasite to the drug as a cause of resistance. This latter point has been dealt with by Yorke *et al.* (1931) and by Hawking (1937). They have shown that live resistant parasites do not absorb the drug.

Certain observations have been made on the respiration of trypanosomes by different authors. Fenyvessy and Reiner (1924) noted that phenol in small

* Beit Memorial Fellow

† Leverhulme Fellow under the Medical Research Council.

concentrations adversely affects respiration, as well as viability of trypanosomes (*T. equiperdum*). KCN has little effect, while quinine in the concentrations used stimulated respiration, and germanin had no action outside the body. Issekutz (1933) found that neosalvarsan and trypaflavin greatly reduced O_2 uptake and destroyed the parasites. He also studied the lethal effect *in vitro* of animals treated with germanin and other compounds. Jancsó and Jancsó (1935) established the therapeutic action of synthalin (decamethylene diguanidine), and Lourie and Yorke (1937) have shown that this drug exerts its lethal effect by direct action on the parasite.

So far as we are aware, no observations have been made in this field with malaria parasites.

MATERIAL AND METHODS

The strain of *P. knowlesi*, as well as the method of isolation and technique of the experiments, was that described by us in the preceding communication (see above, p. 43). The same applies to the normal and arsenic-fast strains of *T. rhodesiense*, for which we are indebted to Professor Yorke. The arsenic-fast strain had been rendered resistant to atoxyl.

The quinine salt used was the monohydrochloride (*purissimus* Howard). For atebrin bihydrochloride and plasmoquine we are indebted to Messrs. Bayer and Co., and for a number of arsenical preparations to Messrs. May and Baker. The arsenic derivatives of camphor dealt with were prepared by Dr. Loudon. Certain organic bases used have been prepared by us, the nature and properties of which will be described elsewhere.

In carrying out the experiments, the 3·0 ml. in the right-hand flasks, except in special cases, consisted of 1·0 ml. standard suspension in serum, 1·0 ml. Ringer (or, in the case of trypanosomes, Ringer with 0·6 per cent. glucose, giving a final 0·2 per cent. in the mixture) containing an amount of the drug to give the final concentration required in the mixture, and 1·0 ml. M/15 phosphate buffer pH 7·4. The left-hand flasks were identical, except that suspension was replaced by serum, and the ' controls ' were normal preparations identical in all respects with the above except that no drug was added, Ringer (or Ringer-glucose) being used in place of Ringer (or Ringer-glucose) containing drug. To ensure rapid and thorough mixing, the mixtures were prepared by measuring out (*a*) suspension and (*b*) Ringer + buffer into two separate test-tubes, followed by rapid mixing of the contents. In all cases, unless otherwise stated, the concentrations given are molecular, and refer to that finally present in the mixture.

Where considered necessary, the mean uptake of three flasks was used, but, in determining the quantitative effect of different concentrations of a drug, we usually employed two only, since the results were invariably very close, and in cases of doubt repeat experiments were performed. Readings were taken at 15-minute intervals, usually for a period of two hours.

The question of the best indication of inhibitory effect has received some consideration. After a good deal of experimentation, we have found the most generally useful to be an inhibition coefficient calculated on the basis of one hour's uptake of oxygen. This coefficient (' h ') is arrived at by taking the difference between the means of the control and the experimental manometers, and expressing this difference as a percentage of the former value. The following example will make this clear :—

Drug. Quinine 0·005. Parasite substance.

Mean uptake for 1 hour in μl. :

Control without drug	225
Flasks with drug..	172
Difference	53

$$\text{Percentage coefficient of inhibition } h = \frac{53}{225} \times 100 = 23{\cdot}6$$

GENERAL CHARACTER OF INHIBITIVE EFFECT

Our results with different compounds are set out in tables, the coefficients of inhibition found at the different concentrations being given.

It will be seen from these tables that some compounds, including organic bases and some arsenical compounds, show no inhibitive effect either with trypanosomes or with malaria parasites, or such small effects at high concentrations (0·001) that these cannot be distinguished with certainty from what may be merely physical effect.

Of those that do inhibit, the effectiveness varies : some are moderately effective, acting at concentrations of the order of 10^{-3} to 10^{-5}, whilst others are much more powerful, acting in concentrations of the order of 10^{-6} or over. These latter have so far been found by us only among trivalent arsenical compounds acting upon normal trypanosomes.

In many cases where the concentration is sufficient, inhibition is absolute from the time of closing the taps. Such an effect, for example, is seen with camphor compound I (camphor-10-arsinic acid) in concentration 1/100,000 against normal trypanosomes. At concentrations below that giving complete and immediate inhibition, the effect is shown by a decreasing inhibition, until at a certain concentration no inhibitive effect is demonstrable.

As will be seen from the final tables, the range between the concentration showing minimal effect and that giving complete inhibition differs considerably in the case of different drugs, not only in its place in the scale of concentration, but also in the range of concentration covered. For example, against normal trypanosomes the zone of minimal effect to complete inhibition for reduced tryparsamide extends from concentration 10^{-7} to 10^{-4}, whereas that for camphor I is from $10^{-6\cdot6}$ to $10^{-5\cdot5}$, and that for quinine, so far as the upper limit has been ascertained, $10^{-4\cdot3}$ to $10^{-2\cdot0}$. The position and range of effectiveness of a drug

against a given organism can, therefore, be indicated by figures for the negative logarithm of the concentrations concerned, e.g., reduced tryparsamide 7·0 to 4·0, camphor 6·6 to 5·5, quinine 4·3 to 2·0.

In all our experiments we have found that, over the range of action of a drug, inhibition increases with concentration. The relationship is not linear, but sigmoid in type. On this account, a small increase of concentration in the zone of minimal effect produces a rapidly increasing inhibition, as will be seen from the tables. As complete inhibition is approached, increasing concentration usually gives only a small increase in the value of ' h.' As yet no definite mathematical relationship has been established. The form of the curve with both trypanosomes and malaria parasites is approximately the same.

A difference has commonly been observed by us between the course in time of inhibition displayed against O_2 uptake by weakly and by strongly inhibiting concentrations. In the former case, the percentage coefficient of inhibition, as worked out for the first 15-minute period, is maintained more or less constantly and without obvious tendency to increase at this level in subsequent periods up to two hours or more. In the second case, the coefficient almost invariably increases in value, the rate of increase usually being proportional to the initial value observed. Usually, where the inhibition is considerable in the first reading (h = 70 or over), complete inhibition is produced within the hour. On the whole, the general impression was obtained that the tendency to rate of increase in inhibition with trypanosomes was greater than that observed for malaria parasites. In part, this was due to the fact that the higher values of inhibition were more commonly seen with the trivalent arsenic compounds acting against trypanosomes, but, apart from this, the massed figures in the tables show something of the same effect.

The following tables give a summary of results for a large number of observations with both types of parasite.

TABLE I

Showing mean coefficient of inhibition over 15-minute periods for all drugs

Trypanosomes

	Percentage inhibition per hour				
	<10	10–	30–	50–	80–
No. of observations	9	15	7	8	20
Time in minutes					
15	5·3	12·5	28·5	39·6	75·1
30	6·6	15·9	36·8	59·9	87·6
45	7·3	16·8	46·5	72·2	93·1
60	6·4	18·0	53·0	85·3	94·8
75	10·3	22·0	53·3	93·4	95·8
90	5·5	24·3	56·9	94·2	95·4
105					97·5

TABLE II
Malaria parasites

	Percentage inhibition per hour				
	<10	10–	30–	50–	80–
No. of observations	3	5	6	5	4
Time in minutes					
15	8·4	29·3	30·6	58·0	71·2
30	6·6	21·0	38·3	69·5	80·8
45	6·0	21·5	42·2	67·3	86·7
60	10·8	24·2	50·8	66·6	89·8
75	12·1	29·0	65·9	69·3	89·2
90	14·3	24·2	68·3	70·2	90·9
105		29·5	75·0		
120		28·8	79·1		

The effect of varying amounts of the organism has been studied by us in the case of inhibition by quinine. The following are the results of experiments obtained using this drug with 1, $\frac{1}{2}$, $\frac{1}{4}$ and $\frac{1}{8}$ the standard suspension. The figures, except in the last two columns, are the coefficients of inhibition calculated for one hour's uptake.

TABLE III

	20.2.37	22.4.37	22.4.37	5.5.37	8.5.37	Mean	Relative inhibition
1. Normal amount (0·036)	10·7	3·8	8·3	4·3	5·8	6·6	1·0
2. Half amount (0·018) ...	17·6		14·6			16·1	2·4
4. Quarter amount (0·009)		21·3				21·3	3·2
8. Eighth amount (0·0045)				24·0	29·0	26·5	4·0

In the case of this drug, therefore, the volume of organism deposit affects regularly the degree of inhibition. The proportionate inhibition for the fractions of standard suspension given above is very approximately respectively 1, 2, 3 and 4, and indicates a logarithmic relationship between amount of substance and inhibition. A similar relation was found by Rona and Bach (1920) to exist between concentration of drug and rate of inhibition of enzyme action.

RELATION OF INHIBITION OF O_2 UPTAKE TO LETHALITY

Our experiments were commenced under the belief that, in studying inhibition of O_2 uptake, we were dealing with inhibition working through respiratory enzymes. It soon became apparent, however, that the question

at issue was how far this was the case. Reduction in oxygen uptake in the case of trypanosomes was clearly associated with reduction in the number of living forms, so that lethality alone, from whatever cause, might have produced the effects.

Assuming, however, that lethality in this case was the outcome of respiratory inhibition, it may be questioned to what extent respiratory inhibition and lethal effect could be separated.

We have already shown (see above, p. 43) how difficult it is, using living suspensions of trypanosomes, to distinguish between these two effects, e.g., where trypanosomes are deprived of sugar. In the case of malaria parasites the difficulties are greater, since there is no ready test available, such as motility, and, though material inoculated into a monkey might give rise to infection, this gave no clue to the proportion of organisms which were viable. Until evidence to the contrary was obtained, it appeared, therefore, that inhibition in the case of malaria parasites must be regarded as probably also closely linked to lethal effect, though we have no data on this point. We have already stated that we have so far been unable satisfactorily to distinguish ' living ' from ' dead ' malaria parasites through vital staining. In each case, the reaction of all parasites was apparently the same, whatever the conditions relative to the dye.

As it seemed desirable to obtain some data correlating inhibition with lethality, an attempt was made in the case of trypanosomes to determine the relation between reduction in the number of living organisms (lethal effect) and reduced uptake, by counting the trypanosomes at intervals over which the O_2 uptake had been measured. Three control flasks were used, containing the same trypanosome suspension as three flasks with drug added. After equilibration for 15 minutes at 37°, counts were made of the number of living trypanosomes in each, using a Thoma-Zeiss haemocytometer, following dilution in a glucose medium, to 1 in a 100, which gave a suitable count of about 50 parasites on the ruled area. At the end of 30 minutes and 60 minutes samples were again withdrawn and counts made. The oxygen uptake was being measured during this time in both control and experimental flasks. The data given in Table IV were obtained.

The method offered several difficulties. In the first place, owing to the drug action parasites became sluggish or motionless, and in varying degrees altered and broken up, and it was difficult always to decide what forms should be included in the count. Again, accuracy of the counts, even after some experience and with every assistance to minimize effort, was not so great as could be desired. Only one count could be relied upon, since a repetition meant examining material at some point after the correct time-interval, and this prevented the use of means of several counts.

Whilst there seemed to be some lag in the case of reduced tryparsamide, i.e., inhibition was greater than the reduction in numbers, we failed in similar experiments with quinine to demonstrate a similar lag, and, moreover, results were not always concordant. It is perhaps to be expected that lessened motility (sluggishness) would in itself diminish O_2 uptake, and, even should a lag be

TABLE IV

Showing O_2 uptake in μl. and parasite counts per μl. of standard mixture at equilibration(0′) and at 30-minute intervals (30′, 60′). The last two columns give the percentage inhibition and reduction in numbers with reference to the controls

Drug	Concentration	Time of observation	O_2 uptake		Count $\times 10^{-3}$		Percentage inhibition	Percentage reduction of count
			Normal	Drug	Normal	Drug		
Reduced tryparsamide	0·000,000,1	0′			44	46		
		30′	152	105	47	51	30·9	0·0
		60′	307	180	45	39	41·4	13·3
Reduced tryparsamide	0·000,000,1	0′			43	38		
		30′	124	76	40	32	43·1	20·0
		60′	249	128	46	23	48·6	50·0
Reduced tryparsamide	0·000,000,2	0′			42	44		
		30′	120	101	39	40	15·8	0
Quinine	0·000,15	0′			63	48		
		30′	179	133	66	53	25·7	19·7
		60′	314	230	64	38	26·6	40·6
Quinine	0·000,1	0′			47	38		
		30′	107	87	35	29	18·7	25·6
		60′	159	131	37	24	17·6	35·1
Atebrin	0·000,015	0′			62	67		
		30′	182	161	76	56	11·5	26·3

shown, it would not necessarily indicate action through the respiratory enzymes, since any form of lethal effect in the degree to cause diminished motility might give similar results. In general, therefore, our observations, which in the circumstances it was decided not to pursue further for the moment, must be regarded as inconclusive.

It cannot be assumed, therefore, that the effects which we have studied are due to inhibition of respiratory enzymes. On the other hand, it is not improbable that the observed lethality is the result of such an action by the drug. It would, for example, be impossible to distinguish the effect upon a suspension of deprivation of glucose from that of a powerful lethal substance, such as reduced tryparsamide. The one is as dramatic in its effects as the other, both as regards O_2 uptake and in action on the parasite as seen under the microscope. Further observations upon the enzyme structure of the organisms dealt with are necessary before discussion as to the nature of the effect observed would possess any value.

It can be said, however, that, whether dependent on enzymes or not, inhibition of O_2 uptake is of quantitative nature, and with characters such as we have elsewhere indicated, i.e., the results are reproducible, they are in definite order and affected by the quantity of parasite material present, and the values for inhibition, when plotted against the logarithm of drug concentration, give fairly characteristic curves.

RELATION OF INHIBITION TO THERAPEUTIC EFFECT

From the data given in the tables, it will be seen that certain substances effective against trypanosomes are effective also against malaria parasites. This is so with the quinine and atebrin group of substances. Trivalent arsenical compounds, on the other hand, which are so effective against trypanosomes, are by no means so markedly effective against parasite substance.

The absence of any lethal effect by pentavalent arsenicals against trypanosomes, observed by many previous authors, extends to a similar lack of effect against parasite substance. Stovarsol, as an example, sometimes used in malaria treatment, as also atoxyl, arsacetin and others of this class, have but little effect against parasite substance.

As regards normal and arsenic fast strains of trypanosomes, the general rule, as shown by Yorke and Murgatroyd (1935), that strains made resistant to one aromatic arsenical are resistant to all organic aromatic arsenicals, was found by us in all cases to hold.

Yorke and his co-workers, testing the effect *in vitro* of a number of trivalent arsenical preparations up to periods of 24 hours, found that against the normal trypanosomes these were lethal at extreme dilutions, whereas in the case of arsenic-fast trypanosomes a much greater concentration of drug was required. Our results with regard to O_2 uptake by the two types of parasite in the presence of drug are exactly similar, and the method which we employ gives, therefore, a speedy and reliable way of telling whether a strain is actually drug-fast. Our strain had been made resistant to atoxyl and was in our experiments resistant to all arsenical compounds tested. The distinction was, however, not so marked with the camphor compound as with other trivalent compounds.

As found by Yorke, an arsenic-fast strain is also resistant to acriflavine. This was also confirmed by us. We found, in addition, that a similar resistance is shown by our arsenic-fast strain to methylene blue. The figures found by the above authors are given first in the series below, and those obtained by us in the second column. It should be noted that our figures are somewhat higher, since the drug effect is measured over a period of only one hour, as against 24 hours by those authors. Moreover, all our results are for molecular concentration, and the dilution which we quote must be divided by 1,000 and multiplied by the molecular weight of the drug to be strictly comparable.

TABLE V

	Concentration necessary to destroy trypanosomes *in vitro* within 24 hours at 37°	Minimal molecular concentration giving reduction in O_2 uptake
Atoxyl	1 : 1,600	1 : 1,000
Reduced atoxyl	1 : 100,000,000	1 : 1,000,000
Arsacetin...	1 : 1,600	1 : 10,000
Reduced arsacetin	1 : 100,000,000	
Tryparsamide	1 : 1,000	over 1 : 10,000
Reduced tryparsamide	1 : 100,000,000	1 : 10,000,000
Stovarsol...	1 : 1,600	over 1 : 10,000
Reduced stovarsol	1 : 6,400,000	
Halarsol	1 : 100,000,000	1 : 10,000,000
Novarsenobillon...	1 : 100,000,000	1 : 5,000,000
Acriflavine	1 : 25,000,000	1 : 1,000,000

An important point in regard to the findings by Yorke and his co-workers is the close resemblance between *in vitro* effects and those *in vivo*, so that it is scarcely possible to avoid the conclusion that the mechanism producing lethality *in vitro* is that which produces destruction of the trypanosomes therapeutically. Inhibition experiments, therefore, in this case have a very obvious relation to chemotherapeutic efficiency.

There are some other indications that inhibition, as observed *in vitro*, to some extent at least is related or runs parallel to therapeutic effect. Atebrin, for example, as an inhibitor is some five times as powerful as quinine, and this is about the relation of dosage and relative effect therapeutically in the two cases. All the recognized anti-malarial drugs tested give inhibition, whilst the amine bases tried were ineffective. Methylene blue and acriflavine appear as powerful inhibitors, just as they are recognized as having therapeutic effect.

That a drug may be equally effective against trypanosomes and malaria parasites *in vitro*, whilst therapeutically it is inactive against one or the other, does not exclude the possibility that the mechanism is the same *in vitro* and *in vivo*, for other possible factors enter in, e.g., distribution of drug in the body tissues. Also, in some cases, drugs, e.g., atebrin, against trypanosomes may on trial actually have some therapeutic effect, though too incomplete to make this of much interest where there are more powerful drugs known against these parasites.

An important consideration is the dilution at which effects are obtained. A distinct effect with quinine against malaria parasites has been obtained up to a concentration of 0·00005 molecular (1/50,000 by weight), and with atebrin up to about 10 times this dilution, or 1/500,000 by weight. This effect is observed

in one hour, so that the question of concentration does not rule out the possibility that the effect seen *in vitro* may be that which is effective therapeutically.

INHIBITIVE EFFECT OF DIFFERENT CLASSES OF COMPOUNDS

Anti-malarial drugs

Under this heading, we include compounds of quinoline or acridine with basic side chains. The following four compounds have been studied.

Quinine. Standard mixtures, as described under materials and methods, have been made with varying concentrations of the hydrochloride, and the effect against parasite substance and trypanosomes determined.

Against malaria parasites this drug began to show minimal effect in concentration of 1/20,000, and 50 per cent. inhibition was obtained with 1/1,000, (range 4·43 — 3+). Against normal trypanosomes an 8 per cent. inhibition was given by 1/20,000, and 70 per cent. inhibition in a concentration of 1/5,000. The effect increased with further concentration to 1/1,000, but without complete inhibition (range 4·3 — 3+). The effectiveness of the drug in both cases is about the same. Against the arsenic-fast strain the effect was equal to that obtained with the normal strain.

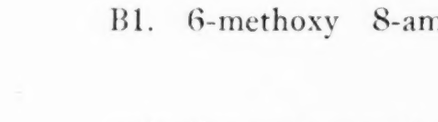
Vuzin. (Iso-octyl hydrocupreine) was used as the bihydrochloride and showed more powerful effect than quinine against trypanosomes, the range being 5 to 3 against both normal and arsenic-fast strains. Vuzin differs structurally from quinine only in that a long aliphatic side chain replaces the methyl of the methoxy group in quinine.

Atebrin. The bihydrochloride was used; minimal effect against malaria parasites was shown at a concentration of 10^{-5}, and against both types of trypanosomes at the same value. At 10^{-4} an inhibition of 85 per cent. was obtained for both types of trypanosomes.

Plasmoquine. One per cent. solutions from ampoules were used. Against both malaria parasites and trypanosomes the effect was approximately that obtained with quinine.

Aromatic and aliphatic amines

We have prepared certain basic substances, some of which represent the side chains of well-known and effective anti-malarial drugs. Both the quinoline nucleus B1 and the side chain B5 of plasmoquine have, for example, been studied separately, in regard to inhibitive effect on oxygen uptake by the two types of parasite. The compounds used were as follows:—

B1. 6-methoxy 8-amino quinoline  represents a quinoline nucleus with simple amino substituent.

B2. Diethyl-amino ethyl chloride ($(C_2H_5)_2N.CH_2.CH_2Cl$), which has provided the side chain in certain synthetic anti-malarials.

B3. 2-amino pentane ($CH_3.CHNH_2.CH_2.CH_2.CH_3$).

B4. 1-diethyl amino 4-chloro pentane $(C_2H_5)_2N.(CH_2)_3.CHCl.CH_3$, where a chlorine atom replaces the amino group of B5.

B5. 1-diethyl amino 4-amino pentane $(C_2H_5)_2N.(CH_2)_3.CHNH_2.CH_3$, which is the side chain present in plasmoquine and atebrin, but with a simple NH_2 group replacing the quinoline and acridine nucleus in the respective compounds.

None of these basic compounds was an effective inhibitor with either malaria parasites or trypanosomes in concentration at which active compounds caused complete inhibition. A small effect was shown against the former, however, by B1 and B2 in concentration of 0·001.

Acriflavine and methylene blue

These two compounds have certain structural features in common. Yorke found—and our experience was similar—that arsenic-fast trypanosomes have a resistance to acriflavine. We have also found that the same holds for methylene blue. With acriflavine, minimal inhibition was obtained at 1/1,000,000 with normal trypanosomes and at 1/100,000 with the resistant strain, which concentration in the case of normals gave an 80 per cent. inhibition.

With methylene blue, minimal effect was recorded at 1/1,000,000 with the normal strain and approximately complete inhibition at 1/10,000. In the case of the arsenic-fast strain, only 27 per cent. inhibition was given at the latter concentration, and almost complete inhibition was shown only at 1/2,000.

Pentavalent arsenicals

We have invariably found that these pentavalent compounds fail to inhibit at any significant concentration (0·001). The compounds of the ordinary type which we have examined are atoxyl, stovarsol, tryparsamide and arsacetin. These have the arsenic directly attached to the benzene ring. We have also used a pentavalent arsenic compound where the arsenic is attached to the side chain at position 10 in the camphor molecule (camphor II). This also was without inhibitive effect, whereas the corresponding trivalent form (camphor I), as shown in the tables, had a very powerful effect.

Trivalent arsenical compounds

All the substances of this type, reduced atoxyl, reduced tryparsamide, halarsol, novarsenobillon, camphor I, tried by us have been extremely powerful inhibitors of O_2 uptake by normal trypanosomes. The most powerful of these has been camphor I, which gives minimum effect at $10^{-6\cdot7}$, the range of action of the drug, however, being shorter than in the next most active compound, halarsol.

As regards effect upon the arsenic-fast strain, all show the same marked lowering of inhibition compared with that obtained with the normal. The

first indication of inhibition is given for most of these substances at approximately 1 in 500,000, but with reduced tryparsamide, even at 1/2,000, the inhibition at this concentration is only 67 per cent. With halarsol complete inhibition is given at 1/10,000, and with camphor I at 1/100,000. This last drug has the greatest effect of all of these compounds against arsenic-fast strains.

Urethane

This ethyl ester of carbamic acid ($NH_2.COOC_2H_5$) is used as a test for the presence of dehydrogenases, whose action it powerfully inhibits. It may be used in high concentration, since any salt effect is absent. We have found that very high concentrations are necessary completely to inhibit O_2 uptake by either malaria parasites or trypanosomes, but as the living organisms were used, and not enzyme preparations, it does not rule out the possible presence of the dehydrogenase mechanism in these parasites.

Whilst the nature of the mechanism of inhibition has as yet been insufficiently studied to enable definite conclusions to be drawn regarding the behaviour of the different classes of substances dealt with, some tentative suggestions may be put forward regarding certain of the relationships observed.

The class of active inhibiting substances represented by the anti-malarial compounds studied are all moderately strong bases (possessing at least one dissociation constant of 10^{-4} — 10^{-7}), and the base, even when these compounds are added as salts, is readily available under conditions of the experiments for combination, e.g., quinine alkaloid and, under appropriate conditions, quinine hydrochloride can form kation complexes with red cell substances (Haffner, 1922). We think it probable that organic bases of this type will usually show inhibition.

The alkyl-amines B1 and B2 have been ascertained to possess extremely low dissociation constants (in both cases approximately 10^{-10}). At pH 7·4 they would be practically completely undissociated, and such may well be a reason for their not inhibiting, i.e., the base cannot exist in the combined form at this pH. The dissociative properties of the remaining bases of this type have so far not been determined.

Though we have not investigated the dissociative properties of the trivalent arsenic compounds employed, it seems likely that in their combining properties these are comparable with the anti-malarial bases, e.g., they are compounds of weak basic character, the combining group in this case not being arsenic but the amine group.

The pentavalent compounds, on the other hand, are arsonium compounds, presumably as in the case of quarternary ammonium compounds forming salts (e.g., sodium salts), the ions of which are both strong, giving complete dissociation comparable to that of neutral salts ; and on account of this property it appears to us that the conditions for the combination necessary for inhibition may be lacking.

TABLE VI

Trypanosomes

Molar concentration of drug	Drug																	
	Quinine		Atebrin		Plasmoquine		Vuzin		Reduced tryparsamide		Novarsenobillon		Acriflavine		Camphor I		Methylene blue	
·0000001									1·9									
2											0·7							
25															0·6			
5									9·7						14·6			
·000001									42·8	2·5	21·3		1·7		53·1		3·9	
2													12·6		67·6	3·4		
3															100	11·3		
5									52·9				13·9		100	51·4		
·00001			6·3		0		8·2		89·1	5·6	93·0		80·0	5·7		100	14·9	10·9
2			50·2	40·3			21·9							19·6				
3			82·9															
4																		
5	8·7			87·5			69·5	71·4						30·8				
·0001	12·9	10·6	84·9		21·8	8·6		90·4	99·1	31·9	97·6			38·0			98·0	27·2
2	67·8																	
3	83·8	86·7																
4	82·0																	
5	85·6				88·9		88·6			67·1								93·8
·001	87·4				94·7		94·7											

Changes produced for any reason in the arsonium ion, as, for example, are known to take place in methylene blue (thionium ion) by reduction, might nevertheless be supposed to bring such substances into the effective range, as Yorke has suggested with regard to the action of pentavalent compounds in the body. A completely ineffective substance of this type might be regarded as one in which not only is the ion strong but it is not easily altered by such processes as reduction. These remarks, we think, may explain the effectiveness of the substances acriflavine and methylene blue, where, even *in vitro* in the last case, the ion is readily subject to reduction.

Thus methylene blue (chloride) is in solution fully dissociated, but by reduction the leucobase is produced. Here conditions comparable to the effectively inhibiting anti-malarial bases may be expected to supervene with combination and inhibition. Thus the organisms themselves, by reduction of the stain, might be the cause of bringing about the combination. Whether compounds in the form of quarternary ammonium bases inhibit, we have not determined, except in the case of atebrin methiodide, which was prepared by us as an example of an actively inhibiting base converted into the ammonium form. This was not active against trypanosomes in a concentration of 0·001, but showed marked action against parasite substance. Tables VI–IX summarize the results of inhibition with malarial parasites and trypanosomes. In Table VI, which is for trypanosomes, the numbers in the drug column represent percentage inhibition, the first being that for normal trypanosomes and the second that for the arsenic-fast strain.

TABLE VII

Malaria parasites (0·1 suspension)

Molar concentration of drug	Drug							
	Quinine	Atebrin	Plasmo-quine	Reduced tryparsa-mide	6-methoxy 8-amino quinoline	Methy-lene blue	Acri-flavine	Atebrin methiodide
·000001				1·7				
5		6·3						
·00001	3·3	9·1	0	26·9		40·5	56·4	
2		23·8						
5	7·5	40·2						
·0001	16·3	61·3	3·6	84·5	4·5	71·0	77·4	44·9
5	26·2	80·3						
·001	46·3		39·0		22·8			79·8

TABLE VIII

The results of inhibition experiments by urethane are given here separately, as the concentrations employed were much higher than in the case of other drugs

Urethane

Molar concentration of drug	Normal trypanosomes	Malaria parasites
0·01	0	0
0·0165		3·2
0·033		10·1
0·066	9·2	18·5
0·132		38·5
0·20	75·5	
0·33	84·9	76·7
0·66	100	100

TABLE IX

Inhibition values for pentavalent arsenicals and bases

Drug	Molar concentration	Trypanosomes		Malaria parasites
		Normal	Arsenic-fast	
Atoxyl	·001	1·3	—	—
Stovarsol	·0001	0	—	1·4
Arsacetin	·0001	1·0	—	—
Camphor II...	·0001	0·2	—	—
	·001	0·9	—	—
6-methoxy 8-amino quinoline	·0001	—	—	4·5
	·001	5·2	1·2	22·8
Diethyl-amino ethyl chloride	·0001	—	0	20·6
	·001	4·3	—	—
1–diethyl amino 4–chloropentane	·0001	—	0	—
	·001	0·3	5·8	8·2
2–amino pentane	·001	3·0	0	5·0
1–diethyl amino 4–amino pentane ...	·001	7·2	—	—

SUMMARY

Certain drugs and compounds have been found at definite concentrations to cause inhibition of O_2 uptake both by trypanosomes and malaria parasites. By employing standard conditions, such inhibition experiments give results which can be expressed as a coefficient of inhibition. While the inhibition always increases with concentration of the drug, no definite mathematical relationship between the two quantities has so far been apparent. But by plotting the logarithm of the concentration against that for the inhibition value, a sigmoid type of curve is obtained.

That the effect is to some degree parallel to therapeutic effect, and may be related to this, is suggested from several considerations. Thus the effects with trypanosomes are exactly parallel to those obtained by Yorke and his co-workers, who have clearly shown that the effectiveness *in vitro* is closely parallel to effectiveness therapeutically. In the case of malaria parasites, the known anti-malarial drugs are effective inhibitors, whilst a number of other bases are not. Methylene blue and acriflavine are effective *in vitro* as they are therapeutically, and in some cases the relative effectiveness *in vitro* appears to be the same as therapeutically, e.g., atebrin is some 5–10 times more effective as an inhibitor than quinine. There is thus the possibility that in some cases at least the inhibition observed *in vitro* is a measure of an identical process taking place in the body when the drug is effective therapeutically.

It would seem that the methods used by us constitute a very delicate means of studying lethality through drug action *in vitro*; in other words, the O_2 uptake gives, so far as can be ascertained, a continuous record of lethal effect by the drug.

The method is rapid, precise and gives repeatable results, and indicates whether a drug has a direct toxic effect. Whether the drug acts in the same way in the body may be complicated by other factors. The method also provides a ready means of telling whether a strain is arsenic-fast. From previous results, the O_2 uptake appears in practice to depend on the organisms being ' alive,' and how far abolition of respiration is equivalent to ' death ' is not quite clear. The two processes seem to be intimately connected.

REFERENCES

EHRLICH, P. (1908). Über den jetzigen Stand der Chemotherapie. *Ber. Deut. Chem. Gesellsch.* (1909), **42**, 17.

FENYVESSY, B. VON, and REINER, L. (1924). Untersuchungen über den respiratorischen Stoffwechsel der Trypanosomen. *Ztschr. f. Hyg. u. Infekt.*, **102**, 109.

HAFFNER, F. (1922). Über den Mechanismus von Hämolyse und Agglutination durch Ionen. *Pflüg. Arch. f. Ges. Physiol.*, **196**, 15.

HAWKING, F. (1937). Studies on chemotherapeutic action. I: The absorption of arsenical compounds and tartar emetic by normal and resistant trypanosomes and its relation to drug-resistance. *Jl. Pharmacol.*, **59**, 123.

ISSEKUTZ, B. VON (1933). Beiträge zur Wirkung des Germanins. Mitteilung I, II. *Arch. f. Exper. Path. u. Pharm.*, **173**, 479.

JANCSÓ, N. VON, and JANCSÓ, H. VON (1935). Chemotherapeutische Wirkung und Kohlehydratstoffwechsel: die Heilwirkung von Guanidinderivaten auf die Trypanosomeninfektion. *Ztschr. f. Immunitätsforsch.*, **86**, 1.

LOURIE, E. M., and YORKE, W. (1937). Studies in chemotherapy. XVI: The trypanocidal action of synthalin. *Ann. Trop. Med. & Parasitol.*, **31**, 435.

RONA, P., and BACH, E. (1920). Uber die Wirkung des Atoxyl auf Serumlipase. *Biochem. Ztschr.*, **111**, 166.

VOEGTLIN, C., DYER, HELEN A., and LEONARD, C. S. (1923). On the mechanism of the action of arsenic upon protoplasm. *Publ. Health Rep.*, **38**, 1882.

——— ——— and MILLER, D. W. (1924). On drug-resistance of trypanosomes with particular reference to arsenic. *Jl. Pharmacol*, **23**, 55.

YORKE, W., ADAMS, A. R. D., and MURGATROYD, F. (1929). Studies in chemotherapy. I: A method for maintaining pathogenic trypanosomes alive *in vitro* at 37° C. for 24 hours. *Ann. Trop. Med. & Parasitol.*, **23**, 501.

——— MURGATROYD, F., and HAWKING, F. (1931). Studies in chemotherapy. V: Preliminary contribution on the nature of drug-resistance. *Ibid.*, **25**, 351.

——— ——— (1935). Biological problems in chemotherapy. *Trans. Roy. Soc. Trop. Med. & Hyg.*, **28**, 435.

ON THE TRANSMISSION OF LEISHMANIASIS

BY

T. SOUTHWELL

AND

A. KIRSHNER

(*Received for publication March 16th*, 1938)

During the last 20 years, investigations on a wide and intensive scale have been carried out with the object of discovering how leishmaniasis is conveyed to man. In 1932, Wenyon, in a paper entitled ' The Transmission of Leishmania Infections,' gave a comprehensive and critical analysis of the work which had been done up to that date.

It is not the intention of the present authors to review the investigations in detail, but we consider that there are sufficient grounds to justify scepticism with reference to the opinions which are generally held regarding the transmission and the stage of the parasite infective to man.

TRANSMISSION IN NATURE BY THE BITE OF THE SANDFLY

In 1924 it was discovered that the Leishman-Donovan bodies of kala-azar developed into leptomonads in the gut of the sandfly. This observation led to the belief that the disease was transmitted by the bite of this insect—an opinion commonly held at the present day.

Shortt, Craighead and Swaminath in 1928 pointed out, however, that in India 3,590 specimens of *P. argentipes*, all apparently harbouring the leptomonad, had been fed on white mice, 2,325 on Chinese hamsters, and 1,274 on human volunteers, without a single successful result. In 1930, Shortt, Craighead, Smith and Swaminath stated that, during the investigations in Assam, of 273,467 *P. argentipes* bred in the laboratory, 79,939 had actually been fed on human or animal cases of leishmaniasis. They noted that negative results were obtained in spite of an intensity of exposure to infection very much greater than could ever be obtained in nature. This caused the Kala-azar Commission to consider seriously whether the development in these sandflies had really anything to do with the transmission of kala-azar in India. Similar negative results have been experienced by other workers in various parts of the world.

The experiments numerically indicated above represent only a part of the research which has been undertaken by a large number of workers on man, hamsters, mice and dogs. Some reasons for the failures have been explained as follows :

1. Man and animals are stated to be highly resistant. In this respect Knowles (1929) remarked that : ' It would appear . . . that the occurrence of

some disease which reduces the patient's resistance leads to the onset of kala-azar . . . kala-azar is essentially a disease of asthenic individuals.' As a result, this author observes: 'If this hypothesis is true, then it appears to me useless at this juncture to carry out further sandfly feeding experiments.'

2. It has been pointed out that the incubation period may be a very long one, and that the results of an infective bite may not become apparent for a year or even more.

3. It is believed that there may be different strains of leishmania, some readily developing in man, whilst others fail to establish themselves.

4. As noted below, the failure to transmit Indian kala-azar by the bite later led to the belief that transmission occurred as a result of infected sandflies being crushed on the skin.

Much of the work appears to have been initiated and continued with the express object of proving a fixed idea—namely, that the disease is transmitted to man by infected sandflies, which inoculate leptomonads whilst biting.

It is maintained that the Mediterranean form of kala-azar is transmitted by the bite of *P. perniciosus*, for in this sandfly the leptomonads, which develop in the intestine, extend anteriorly into the mouth-parts.

The fact that thousands of experiments with infected sandflies have given negative results seems to us to indicate that the opinions at present held in regard to the transmission of *leptomonads* by the bite (or by crushing) of infected sandflies may not be correct.

INFECTIVITY OF LEISHMAN-DONOVAN BODIES

It is known that, if Leishman-Donovan bodies from an oriental sore are inoculated into man or into certain healthy laboratory animals, an oriental sore usually develops, although in rare cases the resulting infection may be systemic. This fact is made use of in certain parts of the world, where, in endemic areas, local lesions are induced on some covered parts of the body, in order to produce an immunity, so that the sores will not occur on the face. Similarly, intraperitoneal inoculation of Leishman-Donovan bodies from a kala-azar patient into a hamster usually results in the latter developing an infection. It is also known that if an animal is fed on spleen pulp containing Leishman-Donovan bodies the animal may become infected.

Shortt, Craighead, Smith and Swaminath (1928) infected one hamster by dropping liver and spleen emulsion containing Leishman-Donovan bodies into its mouth, and later (1929) infected 8 out of 10 hamsters by the same route.

Young, Hertig and Liu (1929) did over a thousand experiments with hamsters, most of them being injections of spleen or liver emulsion into the peritoneum. They found that minute doses produced infection. Scarification gave 18 positive results out of 63 experiments.

Khaw (1930) obtained 12 infections out of 14 experiments, by administering spleen emulsion orally.

We thus see that there is no doubt whatever of the infectivity of Leishman-Donovan bodies to both man and animals. Furthermore, the Leishman-Donovan body is infective, not only when inoculated, but also when administered orally to animals. This latter fact may be of great importance, since Leishman-Donovan bodies have been demonstrated in the nasal discharges of man.

Forkner and Zia (1935) have shown that the nasal secretions of kala-azar cases frequently contain Leishman-Donovan bodies, and they suggest that this fact may have some bearing on the transmission of the disease. Although these discharged parasites are probably not capable of remaining viable for more than a day, there is no reason to doubt that they are as infective to man as they have been proved to be to animals. The possibility that these Leishman-Donovan bodies may be transmitted directly from man to man (by nasal discharges) has been lightly dismissed by a number of workers. The liability to continuous exposure from such discharges over a period of years, under conditions of overcrowding universally met with in the poorer classes, suggests to us that insufficient attention has been directed towards this possible method of transmission. The proximity experiments of the Kala-azar Commission (Shortt, Smith and Swaminath, 1932) with hamsters, in which two out of six became infected, would support this contention.

EXPERIMENTS WITH INFECTED EMULSIFIED SANDFLIES

In addition to the work which has been done with the object of producing leishmaniasis by the actual bite of the sandfly, a very large number of other experiments have been undertaken in further efforts to prove that the leptomonads which develop in the mid-gut of the sandfly are infective. Infected sandflies have been emulsified and (1) applied to scarified skin and (2) inoculated subcutaneously or intraperitoneally. In a number of cases infections have resulted. Thus, the Sergents, Parrot, Donatien and Béguet (1921) sent specimens of *P. papatasii* from Biskra, where oriental sore is rife, to the Pasteur Institute at Algiers. These flies were emulsified in saline and applied to scarified human skin. Two months later an oriental sore developed at the site and Leishman-Donovan bodies were identified in the lesions.

Adler and Theodor (1925) obtained similar results in Palestine.

Aragão (1922) in Brazil emulsified sandflies which had fed three days before on sores, and inoculated the emulsion into the nose of a dog; later, a sore developed, in which Leishman-Donovan bodies could be identified.

Shortt, Barraud and Craighead (1927) inoculated intraperitoneally 53 mice with an emulsion from *P. argentipes* which had been fed on *L. donovani*, and one mouse became infected.

Young and Hertig (1927) reported that flagellates from two species of sandflies inoculated into the peritoneum of hamsters resulted in an infection.

Hindle and Patton (1927) inoculated four hamsters intraperitoneally with flagellates from *P. sergenti*, and all four became infected.

Similar results were recorded by Hindle (1928) and by Adler and Theodor (1931).

The above represent but a few of the experiments which have been undertaken, and it is quite clear that infections do result in experimental work of the above nature. We are by no means convinced, however, that in these cases the infections resulted from the leptomonads present in the intestine of the sandfly, and not from Leishman-Donovan bodies (see below).

Adler and Theodor (1926), dealing with the developmental forms found in the mid-gut of *P. papatasii*, state that ' The parasites show an extraordinary degree of polymorphism which renders their classification difficult. They may be divided into two distinct groups, one being a-flagellar and the other possessing a flagellum.' There are three forms without a flagellum, namely :—

' 1. Leishmaniform bodies with one or two nuclei and one or two blepharoplasts. These bodies were found both in the stomach and, in one instance, in a smear of the upper part of the cardia. . . .

' 2. Leishmaniform bodies, 3μ by 4μ, containing a single nucleus, a blepharoplast and a rhizoplast. These bodies were found in one instance in a smear of the upper part of the cardia containing the oesophageal valve. . . .

' 3. Large round or oval parasites, 5μ to 12μ by 5μ to 8·5μ . . ., with a varying number of nuclei and blepharoplasts . . . found in a smear of the upper end of the cardia.

' None of the above a-flagellar forms which were observed possessed a cyst wall.'

It is extremely unlikely that any of these a-flagellated bodies would ever be transmitted by the bite, for it appears that they are confined to the intestine, and it is only the flagellate forms which are found in the mouth-parts.

Having regard to the above facts, it seems more reasonable to believe that in such cases the infection might easily result from Leishman-Donovan bodies present in the mid-gut of the infected sandfly.

INFECTIONS BY CULTURES

We would now call attention to certain experiments which have been made with cultures containing leptomonads obtained from cases of leishmaniasis.

Napier, Smith and Krishnan (1933) detail the results of a large amount of work undertaken to determine the relative infectivity of Leishman-Donovan bodies and of the leptomonads from cultures of Leishman-Donovan bodies. We are, at the moment, not interested in the results of the infections with Leishman-Donovan bodies, but we may note in passing that, out of 73 hamsters, 45 became infected and in 18 no infection resulted. The experiments to infect hamsters with cultures containing leptomonad forms were divided into five groups as follows :

1. Twenty-seven hamsters were fed by the mouth ; 22 became infected and 5 were negative.

2. Thirty-four were inoculated subcutaneously ; 33 proved positive and 1 was negative.

3. In 9, the skin was scarified and the leptomonad emulsion was applied ; 8 proved positive and 1 negative.

4. In 10, a leptomonad emulsion was inoculated intraperitoneally ; they all became infected.

5. In 9, the parasites were applied to the conjunctiva ; all proved to be negative.

In the above experiments it will be seen that 73 hamsters became infected out of a total of 89.

If the conjunctival route is eliminated, there were only 7 negative results out of 80. In all cases the flagellates were from cultures and ' These were first sub-cultures, 12 days old, on N.N.N. medium.'

Mathewossjan and Zaturjan (1933) inoculated 32 Armenian hamsters with cultures of various ages and 15 became infected.

It is obvious that the above authors considered that all these infections had resulted from the inoculation of leptomonads, a conclusion with which we cannot agree.

Row (1922) argued that infections by inoculation of cultures resulted from the presence of resistant non-flagellate torpedo and O bodies. In seeking to disprove this conclusion, Shortt, Craighead, Khazan Chand and Swaminath (1927) inoculated five-day-old cultures into white mice and obtained 100 per cent. positive results. They assumed that these five-day-old cultures contained none of the a-flagellates described by Row, their assumption being based on the work of Christophers, Shortt and Barraud (1926). These authors, however, stated that it was a significant fact that cultures of the seventh day were the earliest to produce infection in mice, that is, when these a-flagellate torpedo and O bodies of Row were beginning to appear. Furthermore, they note that ' one inoculation of an old culture produced infection whereas repeated inoculations of a culture less than seven days old failed to do so.'

With regard to Shortt, Craighead, Khazan Chand and Swaminath's positive results with five-day-old cultures, we would point out that Christophers, Shortt and Barraud (1926) state that ' The formation of these bodies is a comparatively late phenomenon in the life of a culture, but the time of their appearance is rather a question of the stage of evolution reached in the culture than of its absolute age, so that the bodies appear earlier in a rich culture than in a poor one.'

Wenyon (1926), writing on the culture of Leishman-Donovan bodies, states that ' As the cultures become old, elongate forms become less numerous, and many rounded, non-flagellate bodies appear which resemble in many respects the original leishmania. Many of these are, undoubtedly, degenerate or dead forms, but the fact that subculture can often be obtained from cultures of this type proves that some of them, at any rate, are living.' Further-

more, Adler and Theodor (1927), in describing the forms found in culture, state that ' After eight days there never is an overwhelming majority of the long forms in cultures. Leishman-Donovan bodies and other forms are always present in large numbers.'

Until it has been proved without doubt that the leptomonad *per se* is infective, we consider that it is quite reasonable to believe that the infections obtained by Napier, Smith and Krishnan were due to the presence of Leishman-Donovan bodies in the cultures ; and, further, the evidence would suggest to us that both experimentally and in nature it is the Leishman-Donovan body which is infective, and not the leptomonad. The evolution of Leishman-Donovan bodies into flagellates, both in sandflies and in cultures, and their subsequent change back to Leishman-Donovan bodies, strongly suggest that these latter are the ' metacyclic ' infective forms.

TRANSMISSION IN NATURE BY CRUSHING INFECTED SANDFLIES ON THE SKIN

Transmission in the Indian form of kala-azar is now presumed to be somewhat different. The bite of the sandfly causes an irritation, and the person scratches the part affected, thus crushing and killing the infected insect. In support of this theory it is pointed out that in *P. argentipes*, which is held to transmit the Indian species, the leptomonads do not invade the mouth-parts as they do in *P. perniciosus*. A similar non-invasion of the mouth-parts occurs in the sandfly (*P. chinensis*) which is believed to transmit kala-azar in China. The likelihood of the disease being transmitted by the bite is said to be small, owing to the fact that the presumed infective forms—namely, leptomonads—do not extend into the mouth-parts. This was held to support the idea that transmission occurred by the crushing of the insect on the skin. Here again it is presumed that the infective stage is the leptomonad.

The fact that negative results have always been obtained from biting experiments, whereas positive results are recorded by inoculation of infected sandflies (which we consider comparable to crushing), would indicate that some form of the parasite is present in the intestine of *Phlebotomus* and apparently absent from the mouth-parts. This we consider to be the leishmanioid bodies common in the mid-gut of the sandfly. For the above reasons we doubt whether transmission by the bite takes place, and agree with Wenyon (1932) when he says that ' some fundamental factor in all these experiments has been omitted or . . . infection may take place otherwise than by the bite.

SUMMARY

On the evidence relating to the transmission of leishmaniasis we draw the following conclusions :

1. It has not yet been proved that infections result by the inoculation of leptomonads from the bite of an infected *Phlebotomus*.

2. In the sandfly, both leptomonad and leishmanioid forms occur in the mid-gut, and similar forms occur in cultures.

3. The positive results obtained by inoculation of infected sandflies, and also by cultures, into man and animals may possibly be explained by the presence of these leishmanioid bodies both in the insects and in the cultures.

4. It appears to us possible that transmission of leishmaniasis occurs as a result of infected sandflies being crushed on the skin, and that the infective stage is the leishmanioid body.

5. Sufficient attention has not been directed towards infected nasal discharges as a method of transmission of kala-azar.

REFERENCES

ADLER, S. (1936). Canine visceral leishmaniasis, with special reference to its relationship to human visceral leishmaniasis. Reprinted from *Rep. 3rd Internat. Congr. Comp. Path., Athens*, 1936.

——— and THEODOR, O. (1925). The experimental transmission of cutaneous leishmaniasis to man from *Phlebotomus papatasii*. *Ann. Trop. Med. & Parasitol.*, **19**, 365.

——— ——— (1926). Further observations on the transmission of cutaneous leishmaniasis to man from *Phlebotomus papatasii*. *Ibid.*, **20**, 175.

——— ——— (1927). The transmission of *Leishmania tropica* from artificially infected sandflies to man. *Ibid.*, **21**, 89.

——— ——— (1930). The inoculation of canine cutaneous leishmaniasis into man, and the behaviour of various strains of *Leishmania* in mice. *Ibid.*, **24**, 197.

——— ——— (1931). Investigations on Mediterranean kala azar. I-IV. *Proc. Roy. Soc., B.*, **108**, 447.

——— ——— (1932). Investigations on Mediterranean kala azar. VI. *Ibid.*, **110**, 402.

ARAGÃO, H. DE B. (1922). Transmissão da leishmaniose no Brazil pelo *Phlebotomus intermedius*. *Brazil-Med.*, **36** (i), 129.

CHRISTOPHERS, S. R., SHORTT, H. E., and BARRAUD, P. J. (1926). The result of intra-peritoneal inoculation of mice with cultures of *Leishmania donovani*. *Ind. Med. Res. Mem.*, **4**, 77.

FORKNER, C. E., and ZIA, LILY S. (1935). Further studies on kala-azar : leishmania in nasal and oral secretions of patients, and the bearing of this finding on the transmission of the disease. *Jl. Exper. Med.*, **61**, 183.

HINDLE, E. (1928). Further observations on Chinese kala azar. *Proc. Roy. Soc., B.*, **103**, 599.

——— and PATTON, W. S. (1927). Sandflies and Chinese kala-azar. [Correspondence.] *Nature*, **119**, 460.

KHAW, O. K. (1930). Transmission of kala-azar to hamsters (*Cricetulus griseus*) by the oral route. *Proc. Soc. Exper. Biol. & Med.*, **28**, 231.

——— (1931). Transmission of kala-azar to hamsters (*Cricetulus griseus*) by feeding them with infected carcasses. *Nat. Med. Jl. China*, **17**, 599.

KNOWLES, R. (1929). The kala-azar transmission problem and the factor of resistance. *Trans. 7th Congr., Far East. Assoc. Trop. Med.*, 1927, **3**, 1.

MATHEWOSSJAN, S. T., and ZATURJAN, A. T. (1933). Experimentelle Infektion des armenischen Hamsters *Cricetulus migratorius* mit *Leishmania donovani*. *Arch. f. Schiffs- u. Trop.-Hyg.*, **37**, 190.

NAPIER, L. E., SMITH, R. O. A., and KRISHNAN, K. V. (1933). The relative infectivity of the two forms of *Leishmania donovani* administered by different routes. *Ind. Jl. Med. Res.*, **21**, 305.

ROW, R. (1922). On reversion of the flagellate form of *Leishmania donovani* and *Leishmania tropica* to the resistant non-flagellate torpedo and O body in culture tubes, and its bearing on the attempts at the search for the transmitter. *Ibid.*, **10**, 476.

SERGENT, EDM., SERGENT, ÉT., PARROT, L., DONATIEN, A., and BÉGUET, M. (1921). Transmission du clou de Biskra par le phlébotome (*Phlebotomus papatasii* Scop.). *C.R. Acad. Sci.*, **173**, 1030.

SHORTT, H. E., BARRAUD, P. J., and CRAIGHEAD, A. C. (1927). Note on the infectivity of the forms of *Leishmania donovani* found in *Phlebotomus argentipes*. *Ind. Jl. Med. Res.*, **14**, 577.

——— CRAIGHEAD, A. C., KHAZAN CHAND and SWAMINATH, C S. (1927). The 'resistant non-flagellate torpedo and O bodies ' of Row seen in old cultures of *Leishmania donovani* in their relationship to the production of infections. *Ibid.*, **14**, 567.

more, Adler and Theodor (1927), in describing the forms found in culture, state that ' After eight days there never is an overwhelming majority of the long forms in cultures. Leishman-Donovan bodies and other forms are always present in large numbers.'

Until it has been proved without doubt that the leptomonad *per se* is infective, we consider that it is quite reasonable to believe that the infections obtained by Napier, Smith and Krishnan were due to the presence of Leishman-Donovan bodies in the cultures ; and, further, the evidence would suggest to us that both experimentally and in nature it is the Leishman-Donovan body which is infective, and not the leptomonad. The evolution of Leishman-Donovan bodies into flagellates, both in sandflies and in cultures, and their subsequent change back to Leishman-Donovan bodies, strongly suggest that these latter are the ' metacyclic ' infective forms.

TRANSMISSION IN NATURE BY CRUSHING INFECTED SANDFLIES ON THE SKIN

Transmission in the Indian form of kala-azar is now presumed to be somewhat different. The bite of the sandfly causes an irritation, and the person scratches the part affected, thus crushing and killing the infected insect. In support of this theory it is pointed out that in *P. argentipes*, which is held to transmit the Indian species, the leptomonads do not invade the mouth-parts as they do in *P. perniciosus*. A similar non-invasion of the mouth-parts occurs in the sandfly (*P. chinensis*) which is believed to transmit kala-azar in China. The likelihood of the disease being transmitted by the bite is said to be small, owing to the fact that the presumed infective forms—namely, leptomonads—do not extend into the mouth-parts. This was held to support the idea that transmission occurred by the crushing of the insect on the skin. Here again it is presumed that the infective stage is the leptomonad.

The fact that negative results have always been obtained from biting experiments, whereas positive results are recorded by inoculation of infected sandflies (which we consider comparable to crushing), would indicate that some form of the parasite is present in the intestine of *Phlebotomus* and apparently absent from the mouth-parts. This we consider to be the leishmanioid bodies common in the mid-gut of the sandfly. For the above reasons we doubt whether transmission by the bite takes place, and agree with Wenyon (1932) when he says that ' some fundamental factor in all these experiments has been omitted or . . . infection may take place otherwise than by the bite.

SUMMARY

On the evidence relating to the transmission of leishmaniasis we draw the following conclusions :

1. It has not yet been proved that infections result by the inoculation of leptomonads from the bite of an infected *Phlebotomus*.

2. In the sandfly, both leptomonad and leishmanioid forms occur in the mid-gut, and similar forms occur in cultures.

3. The positive results obtained by inoculation of infected sandflies, and also by cultures, into man and animals may possibly be explained by the presence of these leishmanioid bodies both in the insects and in the cultures.

4. It appears to us possible that transmission of leishmaniasis occurs as a result of infected sandflies being crushed on the skin, and that the infective stage is the leishmanioid body.

5. Sufficient attention has not been directed towards infected nasal discharges as a method of transmission of kala-azar.

REFERENCES

ADLER, S. (1936). Canine visceral leishmaniasis, with special reference to its relationship to human visceral leishmaniasis. Reprinted from *Rep. 3rd Internat. Congr. Comp. Path., Athens*, 1936.

——— and THEODOR, O. (1925). The experimental transmission of cutaneous leishmaniasis to man from *Phlebotomus papatasii*. *Ann. Trop. Med. & Parasitol.*, **19**, 365.

——— ——— (1926). Further observations on the transmission of cutaneous leishmaniasis to man from *Phlebotomus papatasii*. *Ibid.*, **20**, 175.

——— ——— (1927). The transmission of *Leishmania tropica* from artificially infected sandflies to man. *Ibid.*, **21**, 89.

——— ——— (1930). The inoculation of canine cutaneous leishmaniasis into man, and the behaviour of various strains of *Leishmania* in mice. *Ibid.*, **24**, 197.

——— ——— (1931). Investigations on Mediterranean kala azar. I-IV. *Proc. Roy. Soc., B.*, **108**, 447.

——— ——— (1932). Investigations on Mediterranean kala azar. VI. *Ibid.*, **110**, 402.

ARAGÃO, H. DE B. (1922). Transmissão da leishmaniose no Brazil pelo *Phlebotomus intermedius*. *Brazil-Med.*, **36** (i), 129.

CHRISTOPHERS, S. R., SHORTT, H. E., and BARRAUD, P. J. (1926). The result of intra-peritoneal inoculation of mice with cultures of *Leishmania donovani*. *Ind. Med. Res. Mem.*, **4**, 77.

FORKNER, C. E., and ZIA, LILY S. (1935). Further studies on kala-azar : leishmania in nasal and oral secretions of patients, and the bearing of this finding on the transmission of the disease. *Jl. Exper. Med.*, **61**, 183.

HINDLE, E. (1928). Further observations on Chinese kala azar. *Proc. Roy. Soc., B.*, **103**, 599.

——— and PATTON, W. S. (1927). Sandflies and Chinese kala-azar. [Correspondence.] *Nature*, **119**, 460.

KHAW, O. K. (1930). Transmission of kala-azar to hamsters (*Cricetulus griseus*) by the oral route. *Proc. Soc. Exper. Biol. & Med.*, **28**, 231.

——— (1931). Transmission of kala-azar to hamsters (*Cricetulus griseus*) by feeding them with infected carcasses. *Nat. Med. Jl. China*, **17**, 599.

KNOWLES, R. (1929). The kala-azar transmission problem and the factor of resistance. *Trans. 7th Congr., Far East. Assoc. Trop. Med.*, 1927, **3**, 1.

MATHEWOSSJAN, S. T., and ZATURJAN, A. T. (1933). Experimentelle Infektion des armenischen Hamsters *Cricetulus migratorius* mit *Leishmania donovani*. *Arch. f. Schiffs- u. Trop.-Hyg.*, **37**, 190.

NAPIER, L. E., SMITH, R. O. A., and KRISHNAN, K. V. (1933). The relative infectivity of the two forms of *Leishmania donovani* administered by different routes. *Ind. Jl. Med. Res.*, **21**, 305.

ROW, R. (1922). On reversion of the flagellate form of *Leishmania donovani* and *Leishmania tropica* to the resistant non-flagellate torpedo and O body in culture tubes, and its bearing on the attempts at the search for the transmitter. *Ibid.*, **10**, 476.

SERGENT, EDM., SERGENT, ÉT., PARROT, L., DONATIEN, A., and BÉGUET, M. (1921). Transmission du clou de Biskra par le phlébotome (*Phlebotomus papatasii* Scop.). *C.R. Acad. Sci.*, **173**, 1030.

SHORTT, H. E., BARRAUD, P. J., and CRAIGHEAD, A. C. (1927). Note on the infectivity of the forms of *Leishmania donovani* found in *Phlebotomus argentipes*. *Ind. Jl. Med. Res.*, **14**, 577.

——— CRAIGHEAD, A. C., KHAZAN CHAND and SWAMINATH, C S. (1927). The 'resistant non-flagellate torpedo and O bodies ' of Row seen in old cultures of *Leishmania donovani* in their relationship to the production of infections. *Ibid.*, **14**, 567.

SHORTT, H. E., CRAIGHEAD, A. C., SMITH, R. O. A., and SWAMINATH, C. S. (1928). Infection of hamsters (*Cricetulus griseus*) with *Leishmania donovani* by the oral and conjunctival routes. *Ibid.*, **16**, 271.

——— ——— ——— ——— (1929). The infection of hamsters with kala-azar by the oral route. *Ibid.*, **17**, 335.

——— ——— ——— ——— (1930). Third series of transmission experiments in kala-azar with *Phlebotomus argentipes*. *Ibid.*, **17**, 921.

——— ——— and SWAMINATH, C. S. (1928). A brief résumé of recent kala-azar research, with special reference to India. *Ibid.*, **16**, 221.

——— SMITH, R. O. A., and SWAMINATH, C. S. (1932). Transmission experiments in kala-azar by contaminative methods. *Ind. Med. Res. Mem.*, **25**, 79.

WENYON, C. M. (1926). Protozoology, **1**, 411. Lond.

——— (1932). The transmission of leishmania infections. *Trans. Roy. Soc. Trop. Med. & Hyg.*, **25**, 319.

YOUNG, C. W., and HERTIG, M. (1927). Kala-azar transmission experiments with Chinese sandflies (*Phlebotomus*). *Proc. Soc. Exper. Biol. & Med.*, **24**, 823.

——— ——— and LIU, PAO-YUNG (1929). The kala azar transmission problem : field and laboratory studies in China. II : Susceptibility of various rodents to infection with *Leishmania donovani*. *Amer. Jl. Hyg.*, **10**, 183.

STUDIES IN CHEMOTHERAPY*

XVII.—THE ACTION OF UNDECANE DIAMIDINE IN MALARIA

BY

F. GLYN-HUGHES,

E. M. LOURIE

AND

WARRINGTON YORKE

(*Received for publication April 8th*, 1938)

In a recent paper (King, Lourie and Yorke, 1937) the results are recorded of examination for trypanocidal activity of a considerable number of guanidines, isothioureas, amidines and amines, with alkyl and alkylene chains. It was found that certain of the diamidines exhibit a powerful trypanocidal action *in vitro*, and that with the most active member of the series, viz., n. undecane 1 : 11 diamidine, it is possible to produce permanent cures in infected laboratory animals.

As undecane diamidine is of entirely different chemical constitution from all known trypanocidal substances, this discovery is of considerable academic interest. It is consequently of importance to ascertain whether this pronounced parasiticidal power is limited to trypanosomal infections, or whether it extends to other protozoal infections such as malaria.

With a view to examining this question, a number of induced malarial infections in neuro-syphilis were treated with the compound in question. The initial difficulty with which we were confronted in this work was in ascertaining what is the largest dose of the compound which can safely be administered to man. The reports to the Medical Research Council on the action of synthalin in diabetes (1927) indicate that this drug can be given orally to man in doses up to 25 mgm. twice daily, for a limited period, without injury to the patient. As our experimental work on laboratory animals has shown that synthalin (decane diguanidine) is at least twice as toxic as undecane diamidine, there seemed to be ground for believing that as much as 50 mgm. of undecane diamidine could be given to man twice daily by the mouth for a limited period. As experimental work had also shown that the minimum toxic dose of the drug when given parenterally was only about half the minimum toxic dose when given orally, it appeared that the safe dose for intravenous injection would be about 25 mgm. twice daily.

These considerations, therefore, led us to the conclusion that the dose in which undecane diamidine could safely be given to man by the intravenous

* This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council.

route was 50 mgm. per diem, and that in which it could be given by the mouth was 100 mgm. per diem. In our preliminary observations on man we employed the drug in half these doses, but, as no ill effect was observed, we soon decided that it was safe to employ the full doses of 25 mgm. twice daily for the intravenous route and 50 mgm. twice daily by the mouth. These dosages have been adopted in all the cases referred to in this paper, except in Case 15, in which 50 mgm. was given once daily by the mouth, in Cases 13 and 14, where the dose was increased to 75 mgm. twice daily by the mouth, and in Cases 1 and 17, where it was reduced on account of the patients' age and weight. The total number of doses given was usually 5. In no instance were any definite

TABLE

Summarizing the results of treating cases of induced malaria with undecane 1 : 11 diamidine

No. of cases	Method of treatment and dose in mgm.	No. of paroxysms before treatment	No. of paroxysms after 1st dose of drug	Day after 1st dose on which blood became negative	Remarks
	A. Simple tertian malaria infected by blood inoculation				
1	12·5 × 2 ; 4 doses intraven.	10	1	3rd	Boy, 8 years
2	25·0 × 2 ; 5 „ „	6	2	3rd	
3	25·0 × 2 ; 5 „ „	2	1	3rd	
4	25·0 × 2 ; 5 „ „	7	1	3rd	
5	25·0 × 2 ; 5 „ „	8	2	6th	
6	25·0 × 2 ; 5 „ „	6	1	4th	
7	25·0 × 2 ; 7 „ „	5	1	3rd	
8	50·0 × 2 ; 5 „ orally	10	1	4th	
9	50·0 × 2 ; 5 „ „	5	1	4th	
10	50·0 × 2 ; 5 „ „	9	2	4th	
11	50·0 × 2 ; 5 „ „	5	2	3rd	
12	50·0 × 2 ; 5 „ „	12	1	4th	
13	75·0 × 2 ; 6 „ „	9	2	6th	
14	75·0 × 2 ; 6 „ „	7	1	5th	
15	50·0 × 1 ; 6 „ „	10	4	7th	
	B. Simple tertian malaria infected by mosquitoes				
16	50·0 × 2 ; 5 doses orally	9	3	4th	
17	40·0 × 2 ; 5 „ „	13	3	6th	Youth, 17 years
18	50·0 × 2 ; 5 „ „	7	2	6th	
	C. Quartan malaria infected by blood inoculation				
19	25·0 × 2 ; 5 doses intraven.	11	1	6th	

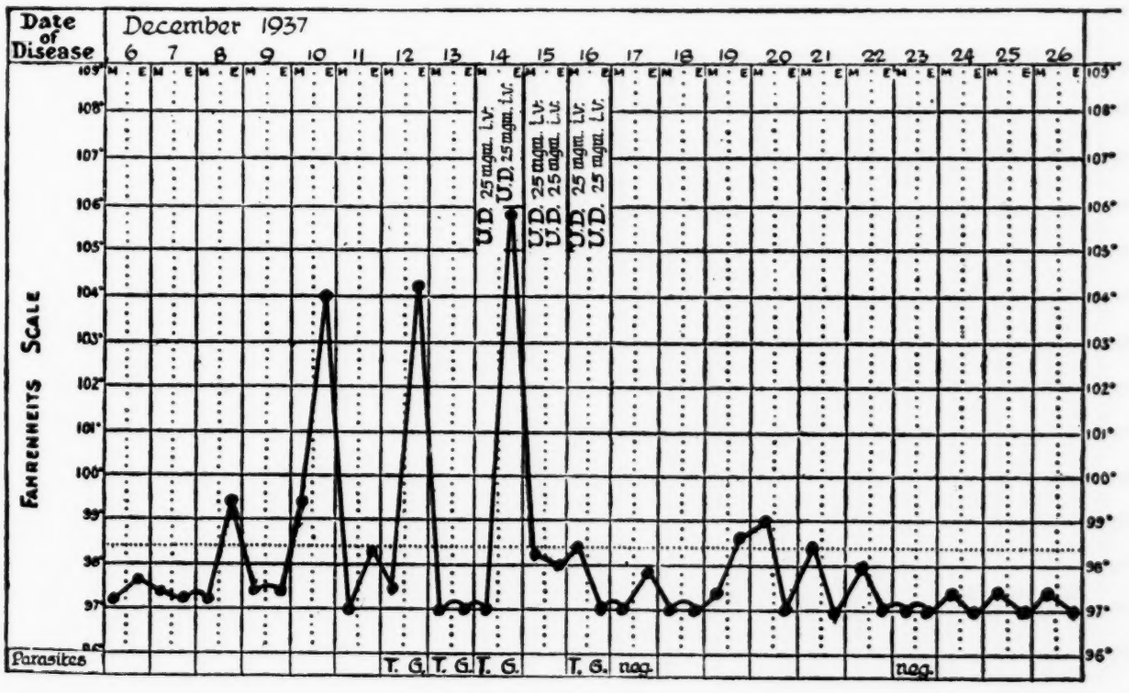
Note.—' 12·5 × 2 ; 4 doses intraven.' implies that a dose of 12·5 mgm. was given intravenously twice daily and that in all 4 doses were given.

ill effects noted, but, as the cases which received 75 mgm. twice daily (Cases 13 and 14) exhibited anorexia and complained of nausea, it appeared that this represented the largest doses which could be given with safety. There was no change in the blood sugar, urea or N.P.N. or in the urine of these cases during treatment.

Details of the results of treating 18 cases of simple tertian malaria and a case of quartan malaria by undecane diamidine are summarized in the table. It will be seen that, of the 15 cases of simple tertian malaria infected by blood inoculation, 7 were treated by intravenous and 8 by oral administration of the drug. In all the 3 cases infected by the bites of mosquitoes the drug was given by the mouth. In the quartan case the drug was given intravenously.

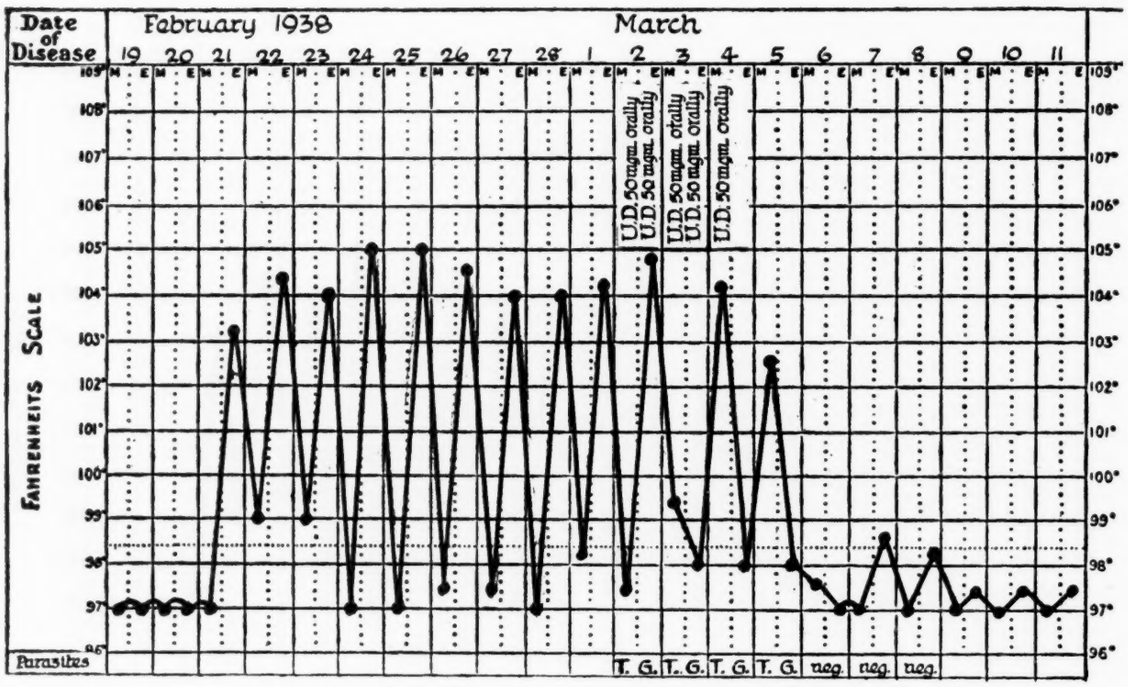
The number of malaria paroxysms which the patient had experienced before treatment commenced varied from 2 in Case 3 to 13 in Case 17. Microscopic examination of the blood showed that all the patients were heavily infected at the time when treatment was commenced. The results of treatment can be summarized by stating that in all cases the administration of undecane diamidine was followed by a cessation of the febrile paroxysms and a disappearance of parasites from the peripheral blood (see charts of Cases 3, 16 and 19).

CASE 3

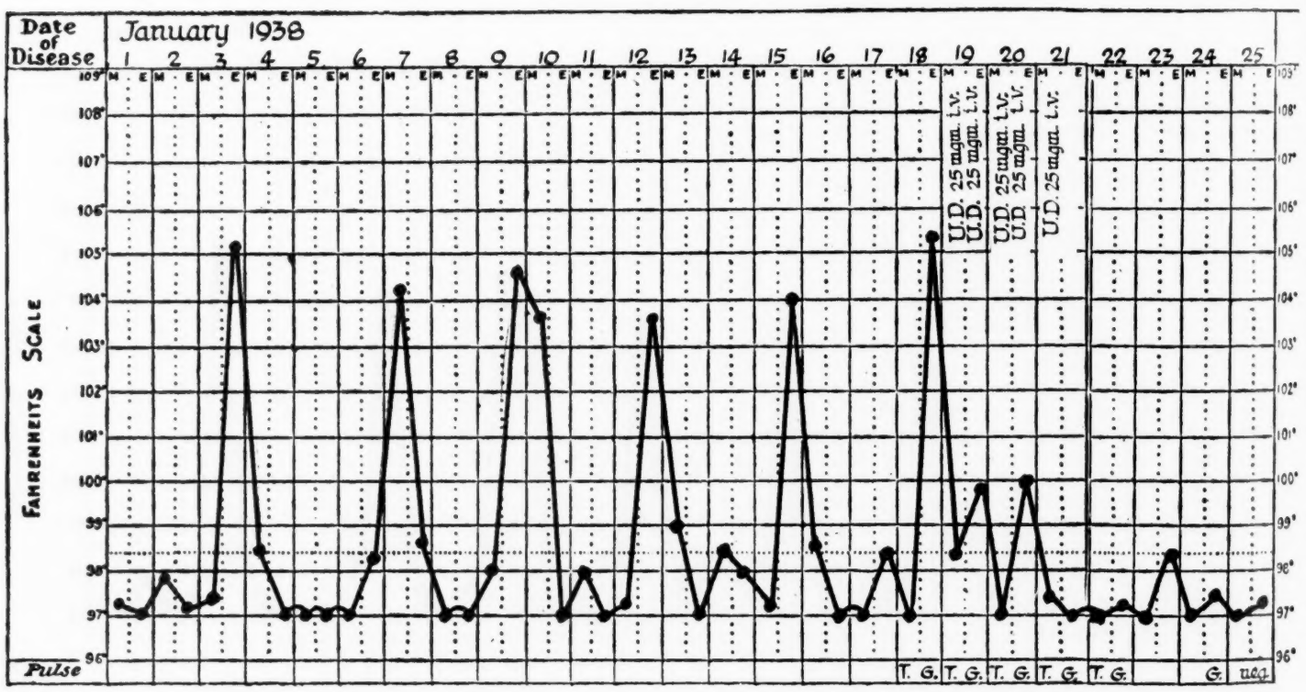

Date of Disease
December 1937
6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
FAHRENHEITS SCALE
U.D. 25 mgm. I.V.
U.D. 25 mgm. I.V.
U.D. 25 mgm. I.V.
U.D. 25 mgm. I.V.
U.D. 25 mgm. I.V.
Parasites
T. G. T. G. T. G.
T. G.
neg.
neg.

U.D. = Undecane diamidine. T. = Trophozoites. G. = Gametocytes.

CASE 16


Date of Disease
February 1938
March
19 20 21 22 23 24 25 26 27 28 1 2 3 4 5 6 7 8 9 10 11
FAHRENHEITS SCALE
U.D. 50 mgm. orally
U.D. 50 mgm. orally
U.D. 50 mgm. orally
U.D. 50 mgm. orally
U.D. 50 mgm. orally
Parasites
T. G. T. G. T. G. T. G. neg. neg. neg.

CASE 19


Date of Disease
January 1938
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
FAHRENHEITS SCALE
U.D. 25 mgm. I.V.
U.D. 25 mgm. I.V.
U.D. 25 mgm. I.V.
U.D. 25 mgm. I.V.
U.D. 25 mgm. I.V.
Pulse
T. G. T. G. T. G. T. G. T. G. G. neg.

U.D. = Undecane diamidine. T. = Trophozoites. G. = Gametocytes.

It will be seen from the table that the patients usually experienced one or two febrile disturbances after commencement of treatment ; exceptions to this are Case 15, who received only half the usual daily dose of the drug, and Cases 16 and 17, who were infected by mosquitoes.

Microscopic examination of the blood showed that the parasites disappeared slowly, and that the blood did not become negative until the 3rd to 6th day after commencement of treatment ; the asexual forms generally disappeared before the gametocytes.

It is not possible at this stage to assess the proportion of cases which relapse, as the observation period of the cases first treated is so far only about 4 to 6 months. Up to the present, however, Cases 5, 15 and 16 have relapsed 8 weeks, 6 weeks and 4 weeks respectively after the first treatment.

It must clearly be understood that the object of this note is not to recommend undecane 1 : 11 diamidine as a satisfactory drug for the treatment of malaria, but merely to draw attention to the fact that this substance has a definite action on *Plasmodium vivax* infections. As undecane 1 : 11 diamidine is of entirely different chemical constitution from any known anti-malarial compound, the observations appear to us to be worthy of record.

REFERENCES

KING, H., LOURIE, E. M., and YORKE, W. (1937). New trypanocidal substances. *Lancet*, **233**, 1360.

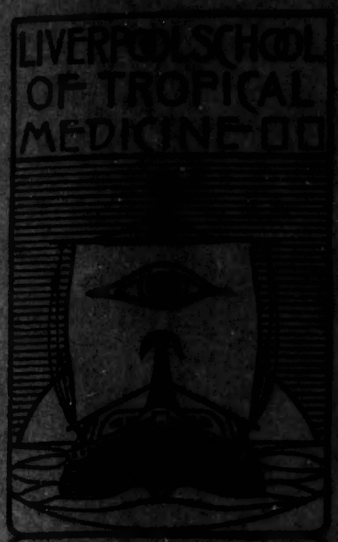
Synthalin in the treatment of diabetes : preliminary reports to the Medical Research Council (1927). *Ibid.*, **213**, 517.

PRINTED IN GREAT BRITAIN BY H. R. GRUBB, LTD., CROYDON.

AUG 25 1938

UNIVERSITY OF LIVERPOOL

2nd August, 1938 Vol. 32. No. 2



Annals of Tropical Medicine and Parasitology

ANNALS
Edited by
Prof. W. Yorke
Prof. D. B. Blacklock
Prof. R. M. Gordon
Dr. T. Southwell
Prof. T. H. Davey

ISSUED BY
THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

THE UNIVERSITY PRESS OF LIVERPOOL

Price Seven Shillings and Sixpence net

Advances in treatment of vitamin deficiency conditions

TRADE MARK **'RYZAMIN-B'** TRADE MARK

RICE POLISHINGS CONCENTRATE

Vitamin B_1 in high concentration. Re-establishes the vitamin supply in the body, particularly of the juvenile type. Beneficial in other cases of Vitamin B_1 deficiency in patients of all ages.

Stimulates the appetite—assists digestion.

Collapsible tubes, 15 gm. (½ oz. approx.) 1/3 each
,, ,, 100 gm. (3½ oz. approx.) 10/- each

TRADE MARK **'TABLOID'** TRADE MARK

NICOTINIC ACID

Believed to be associated with or derived from the Pellagra-preventing factor of Vitamin B_2, which is known to be a complex of tripartite nature. Its value in Pellagra appears to be established.

0·05 gm.
Bottles of 100 tablets, at 4/6

Literature Present to the Medical Profession

BURROUGHS WELLCOME & CO.
LONDON

D 634

A DEVICE FOR APPLYING OIL OR OTHER LIQUIDS TO FLUSHING CISTERNS

BY

D. B. BLACKLOCK

(*From the Department of Tropical Hygiene, Liverpool School of Tropical Medicine*)

(*Received for publication January 28th*, 1938)

The introduction of a piped water-supply and water-carriage sewerage for houses in the tropics constitutes a most important means of eliminating all kinds of disease which depend upon infected human excreta for their propagation. When carefully controlled, it also aids greatly in reducing diseases like mosquito-borne yellow fever, since the spread of these is encouraged in places without a pipe-borne water-supply, owing to mosquito-breeding in the numerous water-containers which are kept in the house and compound.

There are, however, certain risks which may be brought in by a water-carriage sewerage system : one of these lies in the use of the ordinary patterns of waste-preventer cisterns, by means of which the water-closets, urinals and slop-sinks are flushed out ; in such cisterns mosquitoes are apt to breed. Where these types of cisterns are installed, they are often placed in positions in which they are not readily accessible for inspection. Frequently also they are provided with heavy metal or stoneware lids, through which apertures are left for the moving parts.

To prevent mosquito-breeding, an attempt may be made to mosquito-proof all the apertures—a troublesome and not very satisfactory procedure—or to enclose the whole cistern in wire screencloth, leaving only the pull-chain opening to be especially provided for, as by a flexible sleeve. The method most frequently employed is the systematic application of oil to the cistern water. The application has to be repeated frequently, since at each flushing the water is so rapidly siphoned out, almost to the bottom of the cistern, that a large proportion of the oil escapes each time the apparatus is emptied. That this is so can be seen by examining the water seal in the closet-basin after the flushing of an oiled cistern is complete, when a film will be noted on it.

This application of oil is not in itself a difficult matter, and many people make a practice of introducing a few drops from a bottle into the cistern each time after use, or at intervals of some days. But the regular carrying out of the routine requires care and attention, otherwise it may be omitted for longer intervals than are safe.

In order to try to discover a method which would be automatic, depending on the movement of one or other of the working parts each time the chain is pulled, a great deal of experimental work has been carried out.

Several different kinds of tipping contrivances, dropping bottles and pumps have been employed in the endeavour to secure the desired results, namely, the delivery of a definite quantity of oil into the water after each operation of the

lever. The difficulties proved much greater than had been anticipated, largely owing to such factors as the variable range of movement in the corresponding moving parts of different types of cisterns. Manufacturers introduce differences in shape and dimensions into the working parts, and the range of movement

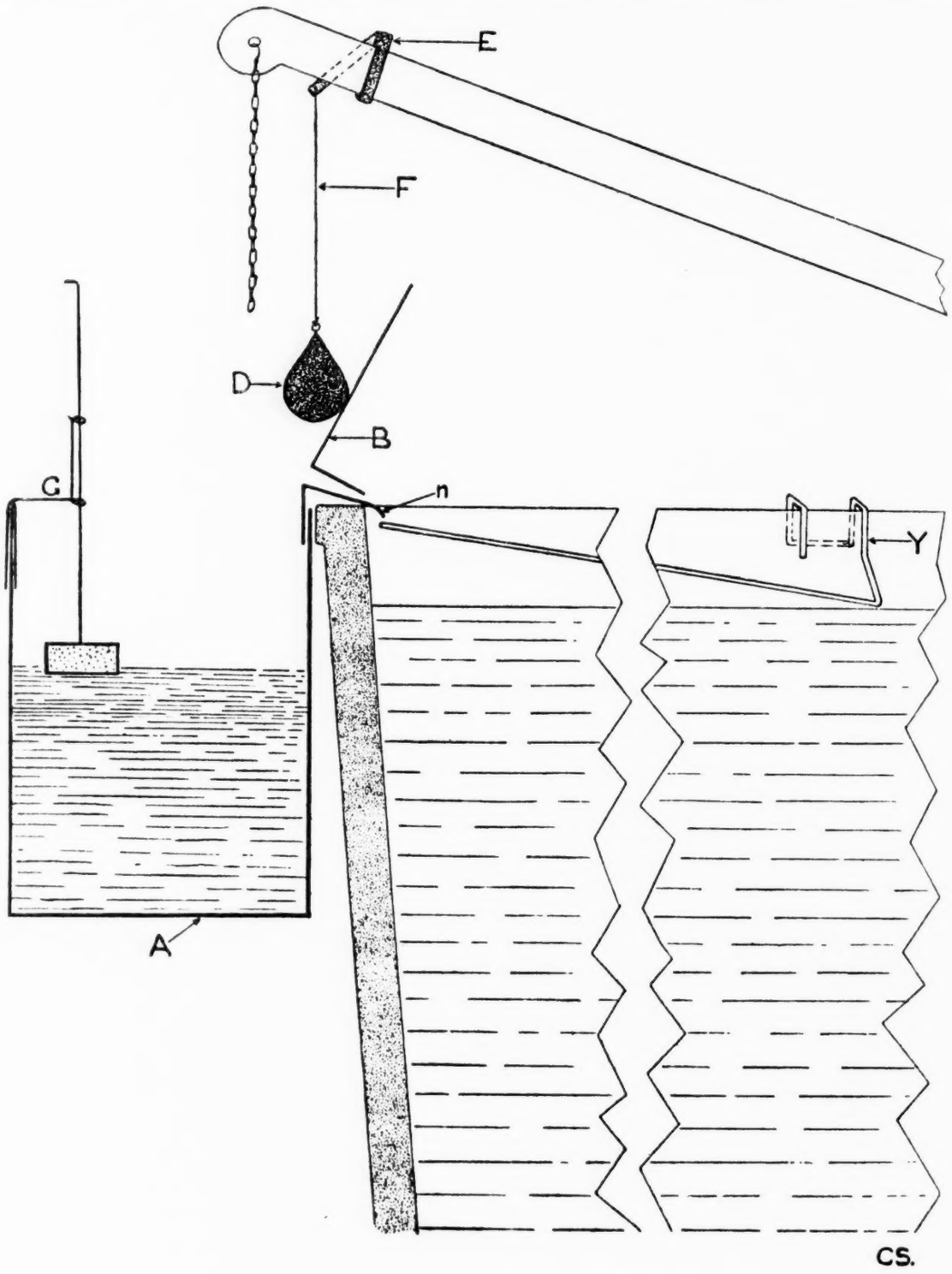


FIG. 1. Sectional view of apparatus.

obtainable varies accordingly. Attempts with small pumps which contained rubber or leather washers or flexible tubing as part of their construction were abandoned after trials, and attention was given to all-metal or glass fittings. Although it was possible to procure glass or metal syringes which would deliver accurate quantities of oil at each impulse of the moving part to which they were applied, the cost was considered too high. A further difficulty was the leakage due to capillarity, a leakage which went on in the intervals when the pump was not working, even in the case of the accurately made and more expensive types. It was thought that advantage could be taken of the very defect of leakage by using the apparatus as a dropper. This, however, proved impracticable, owing to the absence of adjustment of the rate of delivery to suit longer or shorter intervals between the successive operations of the cistern.

In order to be effective, an automatic delivery apparatus not only should apply the quantity of oil desired, but should be so constructed that it delivers the oil, not while the water is sinking in the cistern, but after siphoning is complete and the water-level has again begun to rise ; otherwise a great part of the oil may be lost at once.

The best method of solving the problem which I have so far been able to devise is that described below, which does not involve any expensive appliances.

The method utilizes the up-and-down movement of the lever arm each time the cistern is flushed. The materials needed for the apparatus are as follows :—

Oil-container (fig. 1, A). A rectangular tin, measuring 7 cm. wide, 8 cm. long and 11 cm. deep, was found suitable. The depth of the tin will vary according to the extent of movement of the lever arm, and the length and width according to the space available beside the chain and lever arm when in the down position.

Combined dipper-stop and oil-gutter (fig. 2, B). This was made as follows : A sheet of thin metal $3\frac{1}{2}$ in. $\times$ 2 in. was used. The metal was cut along the solid lines shown in the drawing, and the shaded portions were removed. The whole sheet was then folded along the dotted line *m*, with the result that the lip *n* projected as shown in B_1. The corners formed by *l* and l_1, in apposition after the folding, were bent forward along the dotted lines, so as to keep the run of oil in towards the opening. The sheet was next folded along the line *p* and along the line *r* to the angles shown in B_1. The corners *k* and k_1 were then turned forward to form a gutter leading inwards.

Clips. Two clips (fig. 3, C), ⌊-shaped, with a tightening screw threaded in the longer arm, were used for clamping both the oil-container (A) and the gutter (B) to the end wall of the cistern, at a point just clear of the chain. The portions of the gutter-fitting which were included under the clips are indicated in fig. 2, B.

Dipper (fig. 3, D). A solid pear-shaped dipper of soft metal was used. The length was 2 cm. and the greatest width 1·5 cm. A small screw-hook to take a string was inserted into the narrow end.

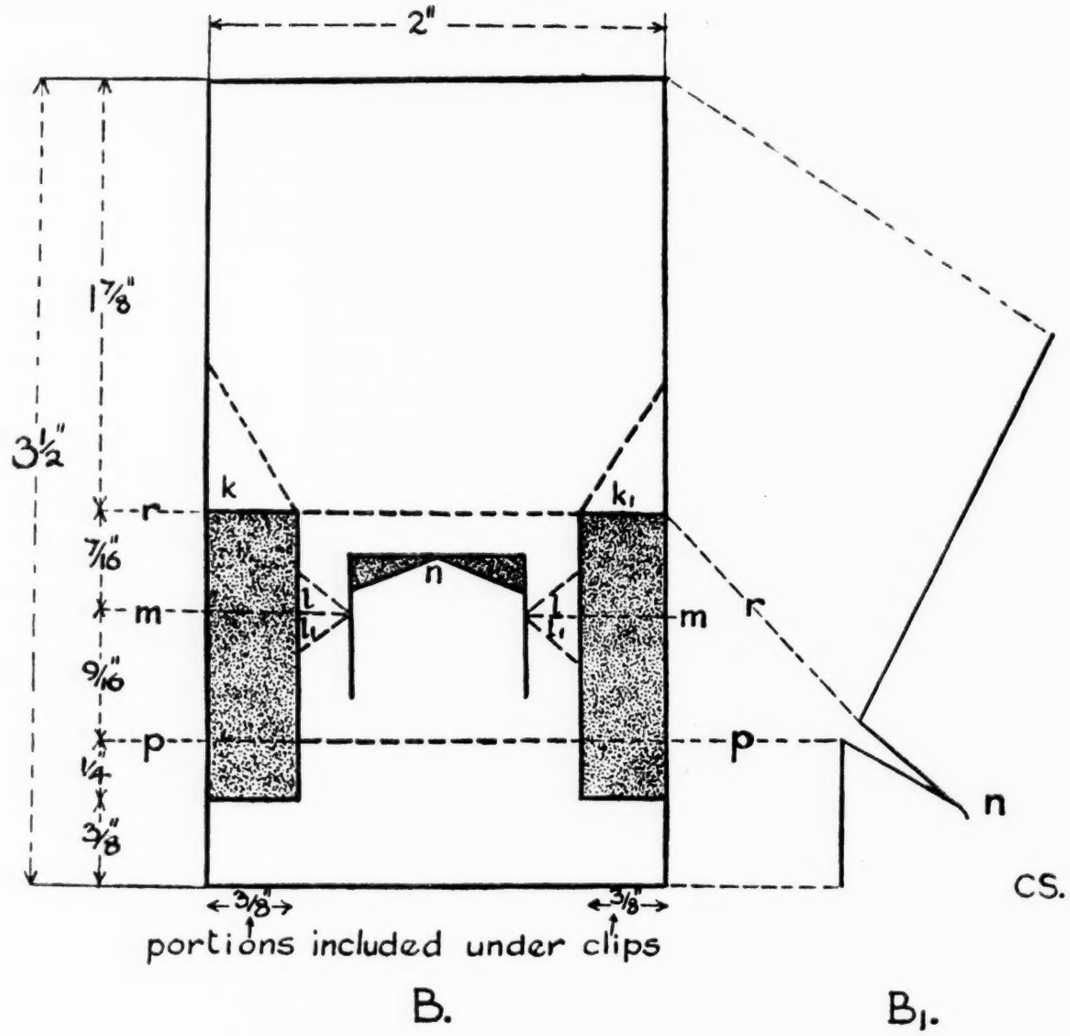


FIG. 2. B.—Cutting and folding metal gutter; B_1.—Sectional view.

Dipper arm (fig. 3, E). A strip of metal, 4 in. × $\frac{1}{4}$ in. × $\frac{1}{16}$ in., to which a string could be securely attached. One end was bent to fit round the lever arm, and holes were drilled through to take a tightening bolt.

Dipper string (fig. 1, F).

Float and guide (fig. 3, G). For indicating the level of the oil in the container. The float was a square piece of cork, $\frac{3}{4}$ in. × $\frac{3}{4}$ in. × $\frac{1}{2}$ in., into which a length of fine straight wire was inserted, the wire being 1 in. longer than the depth of the container. The guide was made with the same gauge wire, by making first a double oval turn in the wire to act as a clip for the side of the oil-container, and then by making two small loops at distances of 1 in. and 2 in. from this clip. The float guide was then bent to form angles, as shown in the diagram.

Chain guide (fig. 3, H). To keep the chain clear of the oil container. A strong piece of wire was bent into two loops, as shown, and secured by a nut and bolt to the lug provided for fitting the lid of the cistern.

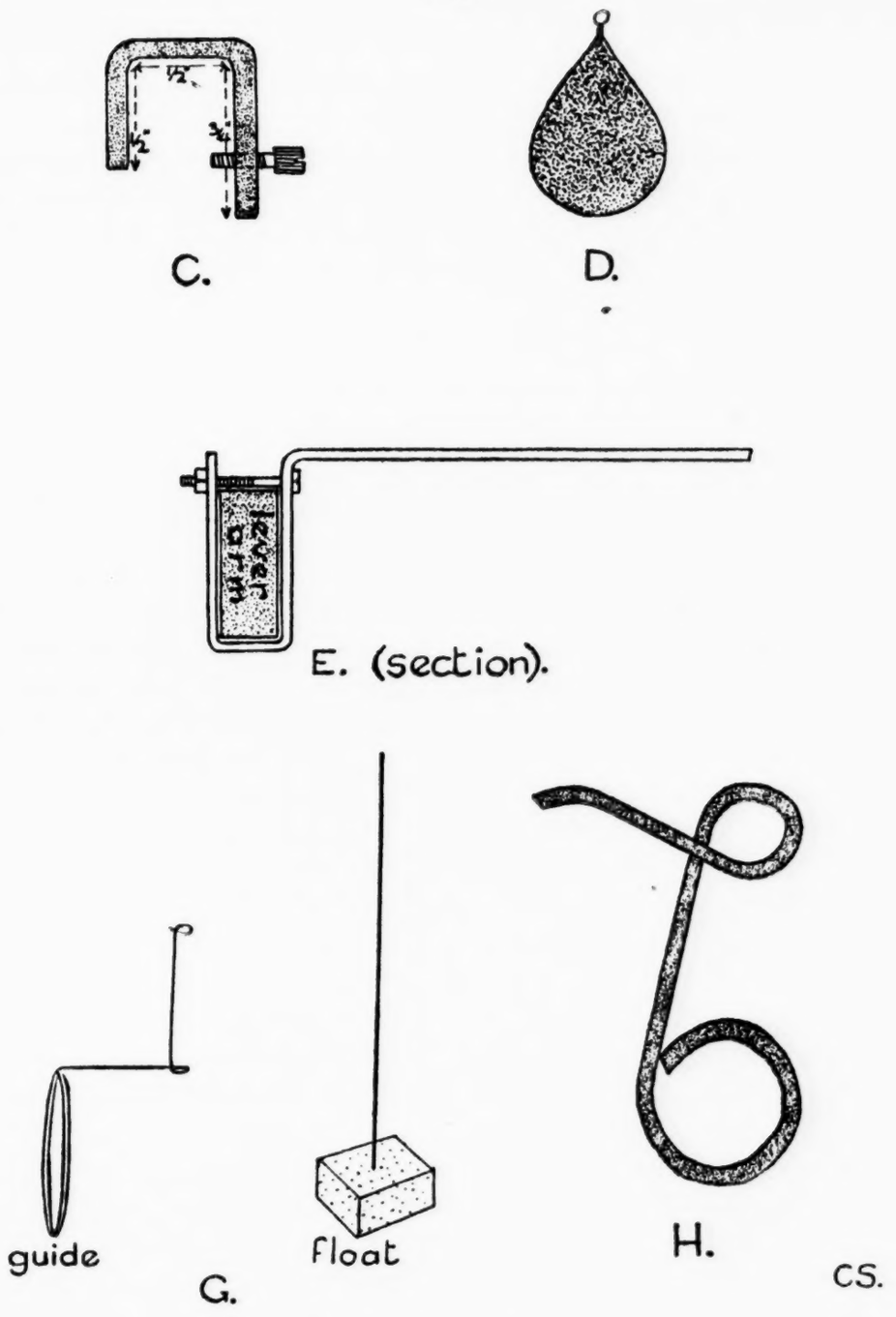


FIG. 3. Various fittings.

METHOD OF ASSEMBLING (shown in fig. 1)

The container (A), together with the combined stop and gutter (B), is attached to the rim of the end of the cistern by means of the two clips. The position of the container is to one side of the lever arm.

The horizontal dipper arm (E) is loosely attached to the lever arm and at right angles to it. The dipper is now suspended from this arm by means of a

length of string (F), so that the bottom of the dipper touches the middle line of the gutter just above the opening in it. The dipper arm is then adjusted and tightened to maintain the dipper in this position.

The guide for the cistern chain can now be fitted, so that it keeps the chain-pull clear of the oil-container. The wire of the oil-float is next passed through the two loops of its carrier, which is then clipped on to the edge of that side of the oil-container which is furthest from the cistern, as shown in fig. 1, G.

Method of Working

When the chain is pulled, the dipper, attached to the lever arm, slides down the gutter and drops into the oil-container. When the lever rises, it pulls up the dipper covered with oil, to the position shown in fig. 1. This oil now drips on to the gutter, passes back through the opening provided, and drops off the lip (*n*) directly on to the water in the cistern.

Oil conveyer (fig. 1, Y). This fitting is only for use in those cisterns in which the lever arm rises before flushing takes place. A length of wire of about 16 S.W.G. can be employed to lead the oil from the lip of the gutter towards the centre of the cistern. It is so bent as to clip on to the edge of the cistern, and can then be cut to such a length as will delay delivery of the oil until the flushing action is completed. The slope is shown in the diagram. A very light metal gutter clipped on to the side of the cistern may be used instead of the single wire. It is adjusted in the same way, to catch the drops of oil from the lip of the gutter, and gives a more satisfactory result.

As regards the quantity of oil or other fluid which can be raised out of the container and delivered into the water in the cistern, this can be regulated very accurately within wide limits. With the plain dipper described above, and using an oil consisting of 88 parts fuel oil, 10 parts kerosene and 2 parts castor oil, 15 drops were delivered at each pull of the chain. By drilling holes in the dipper, or by using a larger one, a quantity up to 2 or more c.cm. can be carried over. In case it is desired to drill holes, the ring should be on the end of a wire running right through the dipper from top to bottom and fitting fairly tightly. The drill-holes, sloping from without inwards, and from above downwards, should reach this central core of wire, so that the oil caught up in the little pockets will escape gradually along the wire to the bottom of the dipper. If the dipper so drilled is found to be delivering too much oil, one or more of the pockets can be plugged up.

The apparatus has been tested with anopheline and culicine larvae and has proved effective in killing them.

ON SOME SPECIES OF *SARCOPHAGA* FROM JAVA AND ITS NEIGHBOURING ISLANDS

BY

CH'I HO*

(*From the Department of Entomology, Liverpool School of Tropical Medicine*)

(*Received for publication April 8th*, 1938)

The following 14 species of *Sarcophaga* have been recorded from Java and its adjacent islands :—

1. *S. taenionota* Wd., 1830.
2. *S. lineatocollis* Macq., 1840.
3. *S. javana* Macq., 1851.
4. *S. dux* Thoms., 1868.
5. *S. albiceps* Meig., 1826.
6. *S. aurifrons* Dol., 1858.
7. *S. fuscicauda* Bött., 1912.
8. *S. orientalis* Park., 1917.
9. *S. knabi* Park., 1917.
10. *S. knabi* var. *flavipalpis* S.-W., 1924.
11. *S. flavinervis* S.-W., 1924.
12. *S. karnyi* Hardy, 1927.
13. *S. antilopoides* Hardy, 1927.
14. *S.* (*Blaesoxipha*) *valgangae* Aldr., 1933.

S. taenionota, *S. lineatocollis* and *S. javana*, like most species of the genus described by the old authors, are unrecognizable. *S. valgangae*, belonging to a genus now considered distinct (*Blaesoxipha*), does not come under consideration. There remain nine species and one variety which have been either recently described or recently reconsidered in the light of modern knowledge.

The species dealt with in the present communication were among a collection of dipterous insects sent for identification to Professor W. S. Patton by Dr. M. A. Lieftinck, Zoölogisch Museum en Laboratorium, Buitenzorg, Java. And the present writer would like here to express his great indebtedness to Professor Patton, for his guidance and for his kindness in affording him the opportunity of studying this material. In all, 62 specimens, comprising 10 different species, were received, of which 28 were females and indeterminable. Three species proved new to science, and one is here recorded for the first time from this region. The nomenclature of one species (*S. flavinervis* S.-W.) is changed, as Senior-White (1924) overlooked the fact that the name is preoccupied. The specific status of three forms is also discussed.

The holotypes of the three new species have been returned to the Buitenzorg Institute, together with their terminalia mounted on celluloid.

* Research Fellow of the China Foundation for the Promotion of Education and Culture.

Sarcophaga antilope Böttcher, 1913 (figs. 1, 2)

SYNONYM : *S. antilopoides* Hardy, 1927.

One male, bearing the label 'W. Java, Bantan, Paling-ping, 19.IV.1935 (M. A. Lieftinck).'

The separation of *S. antilopoides* from *S. antilope* was based on the comparison of the terminalia of the Javanese specimens with Senior-White's drawing of *S. antilope* from India. The present writer has no Indian material for comparison ; but a specimen from Sauter's Collection,* determined as *S. antilope* by Böttcher, and another specimen from the Island of Hainan (which is situated

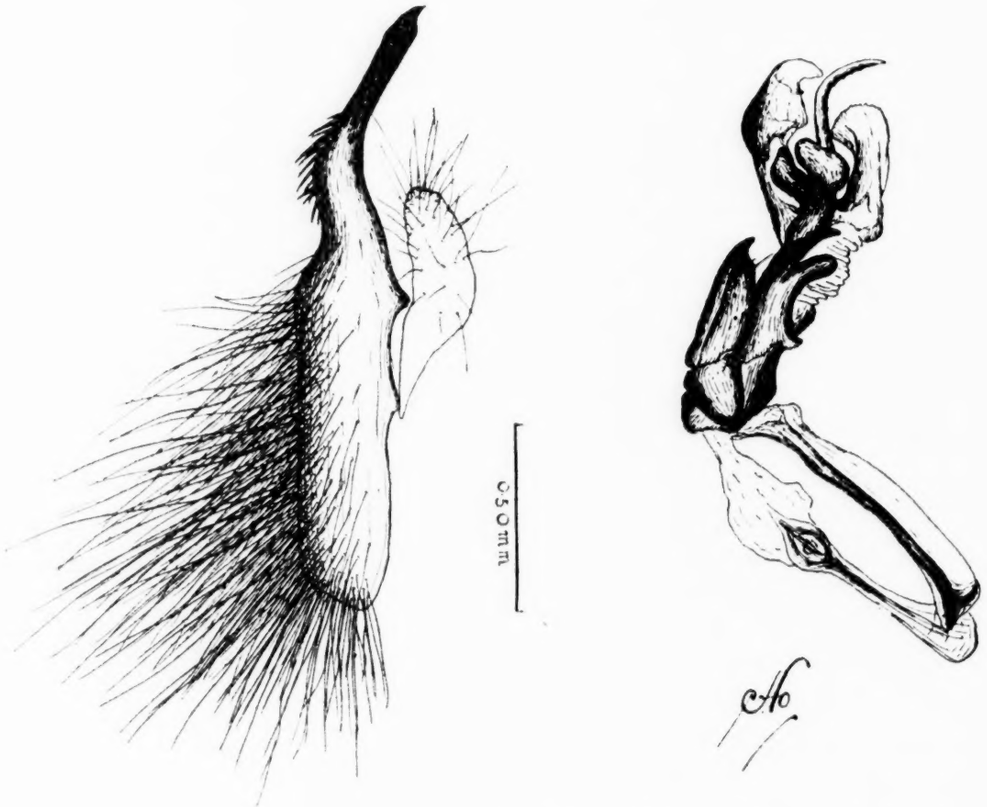


FIG. 1. *S. antilope* Bött. Male terminalia.

about midway between Formosa—the type locality—and Java) were available for study. A careful comparison of the terminalia of these specimens with the example from Java showed them to be identical. It is thus obvious that *S. antilopoides* is a synonym of *S. antilope*. Slight differences in the male terminalia of the same species are often seen in the drawings of different authors ; but such

* The writer wishes to take this opportunity of thanking Dr. W. Horn, Director of the Deutsches Entomologisches Institut, Berlin-Dahlem, for the privilege of examining Sauter's Collection during his visit to Germany last summer, and also for the exchange of a few named specimens.

differences are not always real, and may be due to orientation of the specimen or to a misunderstanding of the structure, and care must be taken in referring to them. In fig. 1 fuller details of the male terminalia are shown. The fourth sternite with a dense patch of black hairs at the posterior end, a character not previously noticed, is also illustrated (fig. 2, *a*).

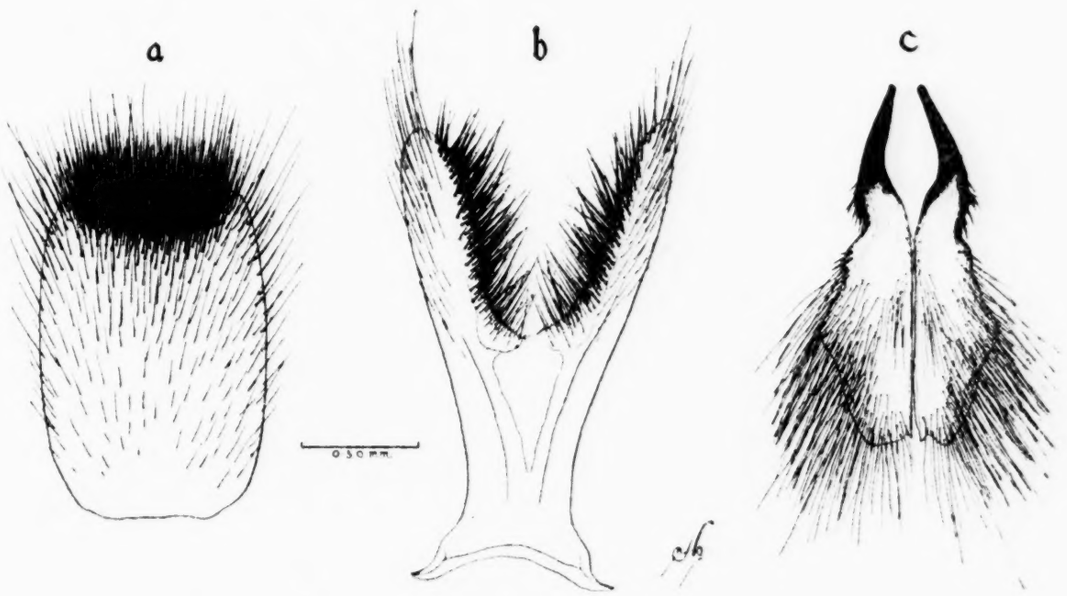


FIG. 2. *S. antilope* Bött. *a*.—Fourth sternite ; *b*.—Fifth sternite ; *c*.—Anal cerci in posterior view.

Sarcophaga senior-whitei nom. nov. (figs. 3, 4)

SYNONYM : *S. flavinervis* Senior-White, 1924 (nec R.-D.).

Two males and four females : Dammerman, Karinou, Djawa, May, 1926 ; and N.-W. Soemba, Laora, 100 m., April, 1925.

A large-sized species, easily recognizable by its striking bright-yellow wing coloration. Squamae and halteres similarly bright yellow. The name *flavinervis* used by Senior-White has been found to be preoccupied in the same genus (Kat. Pal. Dipt., III, p. 483), and must therefore be replaced by a new one. The fourth sternite of this species resembles the preceding one in having a dense patch of black hairs towards the posterior end, but differs from it in that the patch is larger and the hairs are finer and upstanding. This character, together with the forked anterior paramere and a similarity in the general construction of the phallosome, suggests the close affinity of the two species. Females possessing the same striking wing coloration can also be picked out at a glance. Male terminalia, which differ considerably in detail from Senior-White's figures, are here illustrated.

Sarcophaga knabi Parker, 1917

Three males : Dammerman, Doerian, Riouw-Arch., November, 1923 ; and N.-W. Soemba, Laora, 100 m., April, 1925.

Senior-White recorded both this species and its varietal form, var. *flavipalpis*, from Java. But, as pointed out elsewhere (Ho, 1932), the slight colour difference

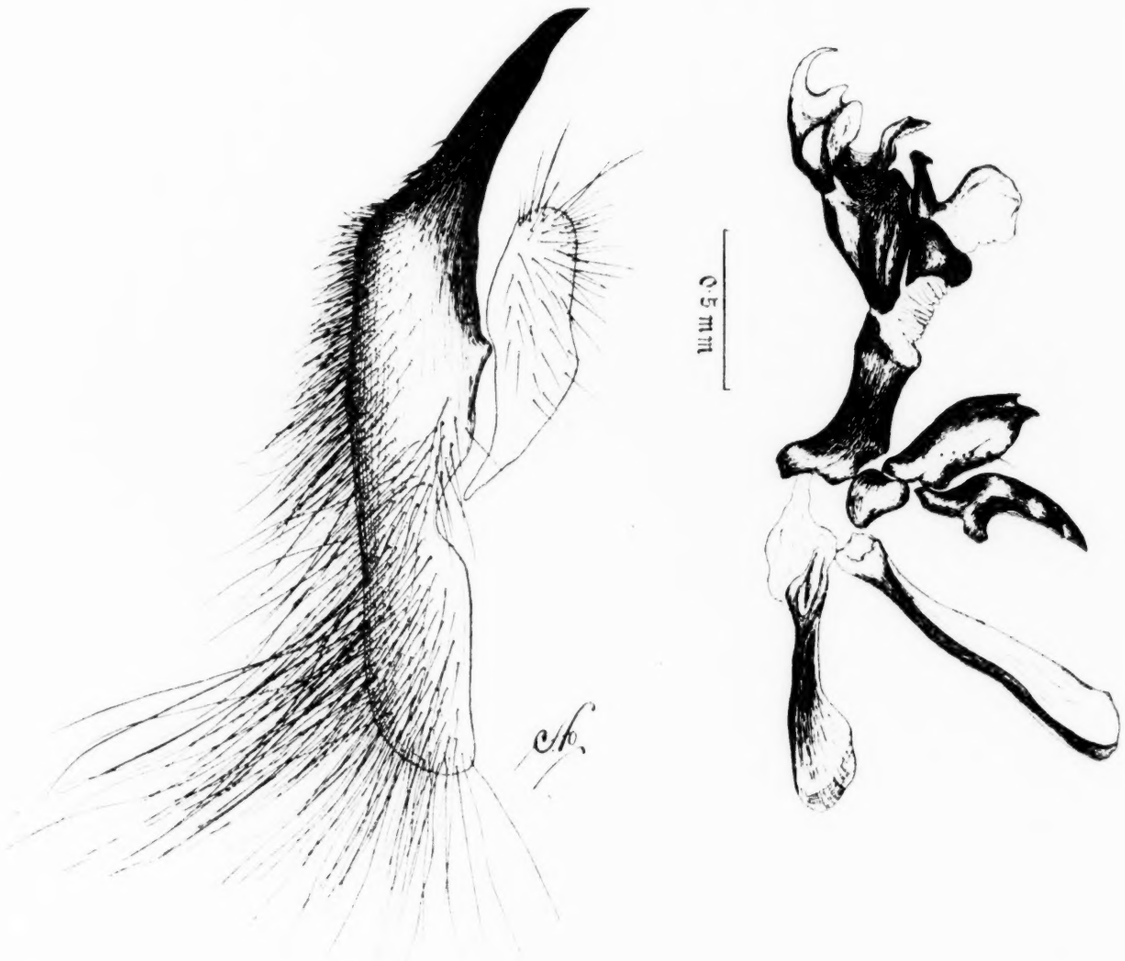


FIG. 3. *S. senior-whitei* nom. nov. Male terminalia.

of the palpi, which is variable, is insufficient to justify varietal status. The three specimens examined showed the palpi much darker in one than in the other two.

Sarcophaga hirtipes var. **orchidea** Böttcher, 1923

One male : Dammerman, Doerian, Riouw-Arch., November, 1923.

This is the first record of this species from the region. The specimen agrees with Böttcher's description of *orchidea* in having a silvery-white face and a black second genital segment. The type-form of *hirtipes*, which is from

Egypt, has a golden face and a red second genital segment. In this connection the writer agrees with Senior-White in considering *orchidea* as a variety and not as a distinct species.

Sarcophaga albiceps Meigen, 1826

Eight males and one female : West Sumatra, Siberut Island and Sipora Island, 1924 (C. B. K. and N. S.) ; Mentawei, September, 1924 (H. H. Karny) ;

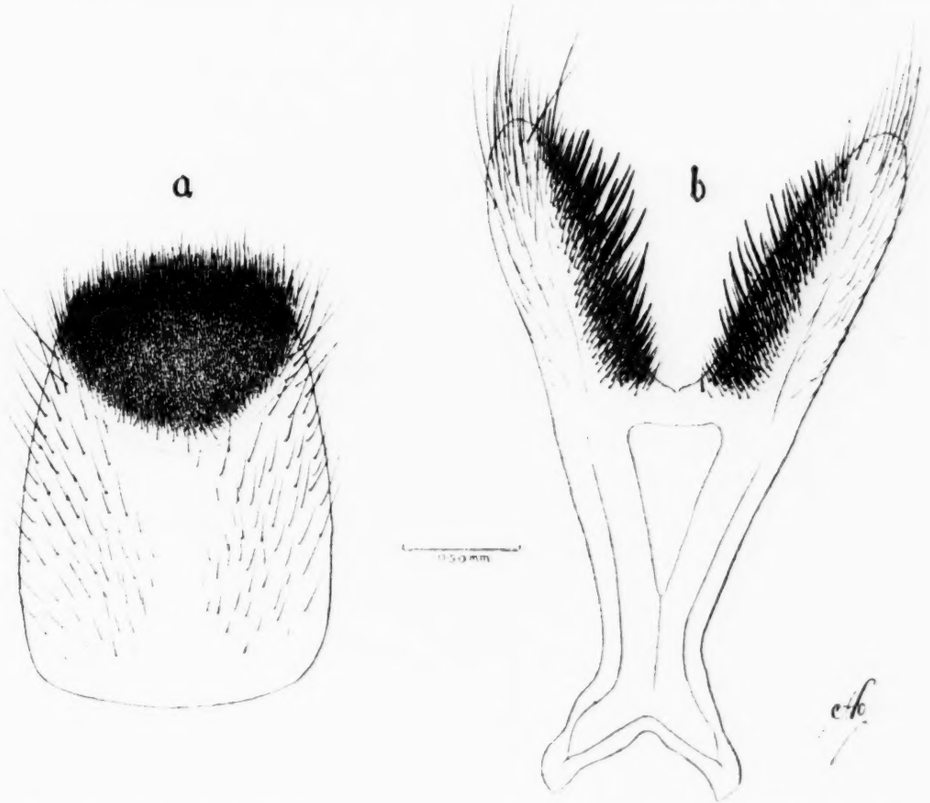


FIG. 4. *S. senior-whitei* nom. nov. *a.*—Fourth sternite ; *b.*—Fifth sternite.

Dammerman, O. Soemba, 450 m., May, 1925 (Mao Marrie) ; W. Java, 600-800 m., Djampang, Tengah, G. Tjisoeroe, March, 1933 (M. E. Walsh).

Apparently one of the most common species in this region.

Sarcophaga fuscicauda Böttcher, 1912

SYNONYM : ? *S. karnyi* Hardy, 1927.

Three males : Dammerman, Doerian, Riouw-Arch., November, 1923 ; W. Java, 600-800 m., Djampang, Tengah, G. Tjisoeroe, January-March, 1933 (M. E. Walsh).

The writer is doubtful about the identification of this species. Hardy (1927) gave the Javanese form a new name (*S. karnyi*). The distinction was

made up after a comparison of this form with the Indian one, and not with the Formosan form. The writer has no example of *S. fuscicauda* from Sauter's Collection for a careful terminalic comparison. An examination of Chinese material, however, showed them to be identical. The three specimens in the present collection showed the second genital segment dark red in two and black in one.

Sarcophaga orientalis Parker, 1917 (figs. 5, 6)

One male: W. Java, 600-800 m., Djampang, Tengah, G. Tjisoeroe, October, 1933 (M. E. Walsh).

Originally described from the Philippine Islands and later recorded from Bali; in both cases a single specimen was taken, and the present specimen is

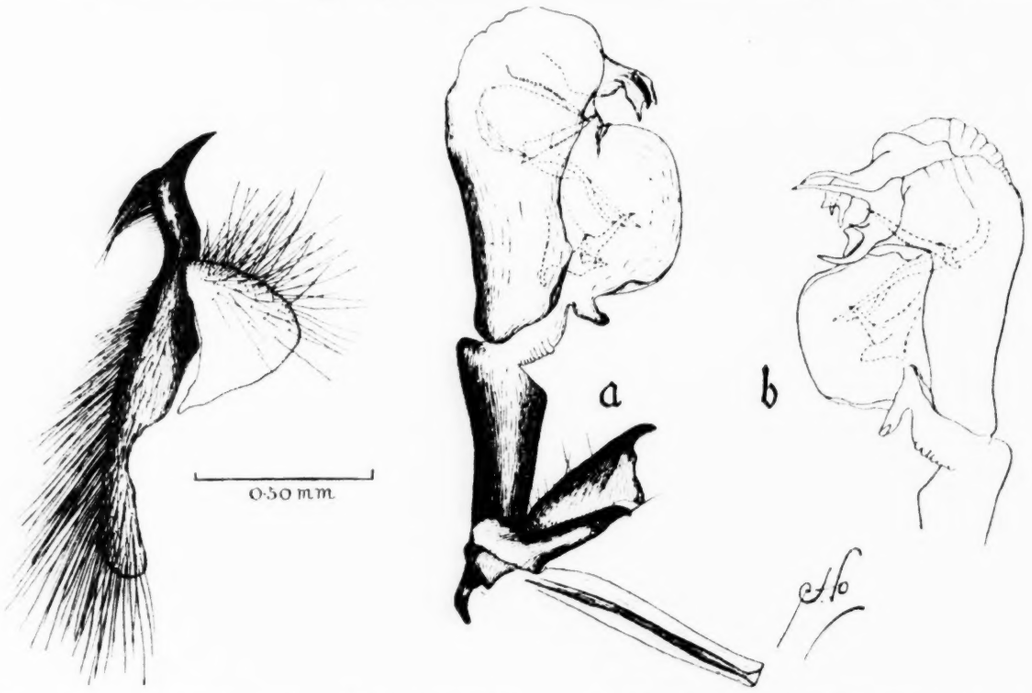


FIG. 5. *S. orientalis* Park. Male terminalia. *a*.—Left view of the phallosome; *b*.—Right view of the same.

only the third to be recorded. An unusual feature of the phallosome of the species is that the processes on either side differ slightly in shape and position (fig. 5, *a*, *b*). Similar asymmetry has also been noticed in the phallosome of *S. orientaloides*, a closely related species. Sternites, unlike those in most species of the genus, are clothed with longer hairs. The other characters agree closely with Parker's description.

Sarcophaga serrata sp. nov. (figs. 7, 8)

MALE. *Head.* Lateral verticals absent. Frons narrow, 0·55 mm. wide or 0·37 width of an eye. Frontal stripe well marked, diverging below, at its narrowest part narrower than two parafrontalia taken together. Parafrontalia and

parafacialia darkish golden, the latter with a row of about 8 longer hairs below, near the eye. Frontal bristles 11 or 12, the lowest three moderately diverging. Antennae black, third segment two and a half times the length of the second

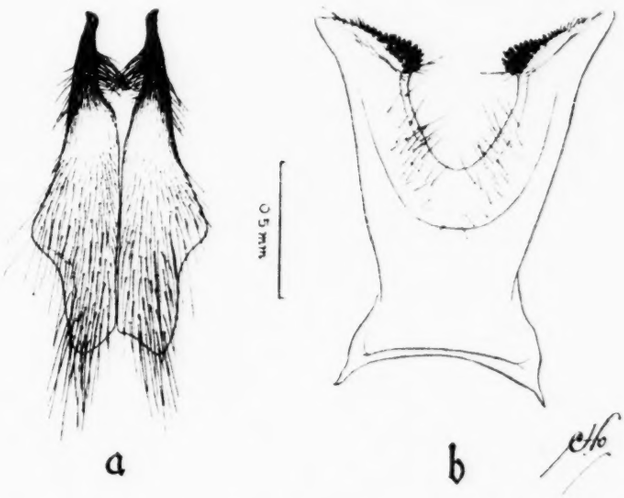


FIG. 6. *S. orientalis* Park. *a.*—Anal cerci in posterior view ; *b.*—Fifth sternite.

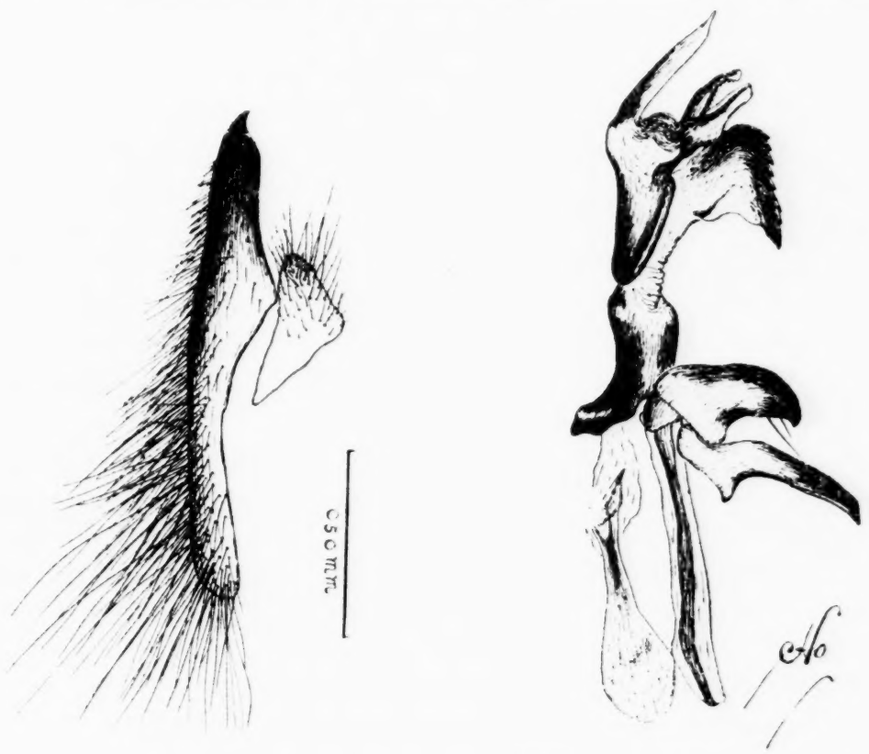


FIG. 7. *S. serrata* sp. nov. Male terminalia.

and reaching almost to the vibrissae, which are at the oral margin ; arista with long plumosity for three-fifths of its length. Palpi black. Bucca about a quarter the eye-height, with black hairs, none pale before the metacephalic suture.

Back of head with three or four rather irregular rows of postocular cilia, pale soft hairs inconspicuously around middle and below.

Thorax. Acrostichals only present as prescutellars. Posterior dorsocentrals 4 or 5, only the last two strong. Intra-alars 1 : 2. Scutellum with the usual two pairs of marginal bristles, one pair of subapicals and one pair of apicals.

Abdomen. Third segment with one pair of lateral marginal bristles, without median marginal bristle. Fourth segment with one pair of median and three or four pairs of lateral marginal bristles. Fifth segment with marginals displaced abruptly by finer ones on ventral edges. Sternites short-haired, fifth sternite as shown in fig. 8, *b*.

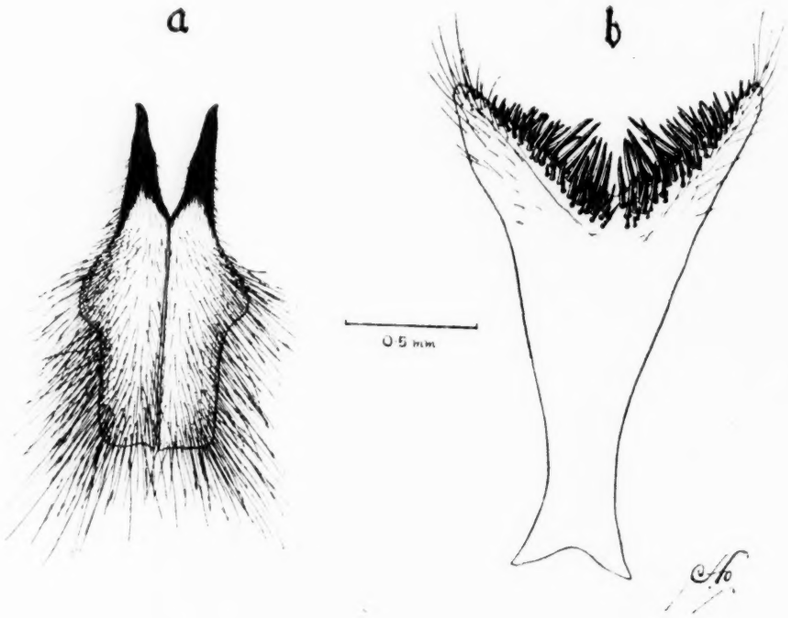


FIG. 8. *S. serrata* sp. nov. *a.*—Anal cerci in posterior view ; *b.*—Fifth sternite.

Wings. Costal spine vestigial or wanting. Section III of costa about equal to the length of section V. Wing membrane hyaline, slightly infuscated along the veins.

Legs. Mid-femur with a fringe and comb ; mid-tibia with one antero-dorsal bristle, without fringe. Hind femur and tibia both fringed on posterior side.

Terminalia. Second genital segment shining brown to black ; first genital segment brownish, thinly grey pollinose, without marginals. Morphological details as shown in fig. 7.

Length. 10·7 and 12·0 mm.

FEMALE. Unknown.

HOLOTYPE. Male, W. Java, 600–800 m., Djampang, Tengah, G. Tjisoeroe, March, 1933 (M. E. Walsh).

PARATYPE. One male, Buitenzorg, July, 1920.

A glance at the phallosome of *S. serrata* and *S. auricauda* will immediately show the close relationship between the two species, although their external characters exhibit considerable differences (see under next species). The general construction of the phallosome of these two species is not unlike that belonging to the *S. albiceps* group, from which it differs in having no prominent anterior membranous bulge on the distal segment of the phallosome, and in the shape and degree of chitinization of the anterior appendage.

Sarcophaga auricauda sp. nov. (figs. 9, 10)

MALE. *Head.* Lateral verticals weak or wanting. Frons 0·67 mm. wide (average of six specimens) or 0·44 width of an eye. Frontal stripe equal to or slightly wider than two parafrontalia taken together, with well-defined and almost straight edges. Parafrontalia and parafacialia golden, the latter with a row of 3–5 hairs below, near the eye. Frontal bristles 11–14, strong, the lowest three or four gently diverging. Antennae black, third segment from two and a half to almost three times the length of the second, and reaching four-fifths of the distance to the vibrissae, which are at the oral margin. Arista with long plumosity for three-quarters of its length. Vibrissal ridge bristly throughout. Palpi black. Bucca almost one-third the eye-height, with black hairs, none pale before the metacephalic suture. Back of head with mostly black hairs, only a little pale soft hair about foramen and below.

Thorax. Ground colour bluish-grey. Three black stripes broader than usual. Acrostichals absent in five specimens, present as weak prescutellars in one. Posterior dorso-centrals 5, only the posterior two strong, the anterior ones hardly recognizable. Intra-alars 1 : 2 or 3. Scutellum with a very broad median black stripe, with two pairs of strong marginal bristles, one pair of weak subapicals and one pair of weak apicals.

Abdomen. Ground colour bluish-grey, with larger black patches than usual. Fourth and fifth segments strikingly golden pollinose, the pollen denser on fifth segment, gradually decreasing on fourth. Third segment with one pair of lateral marginal bristles ; fourth with one pair of median and three pairs of lateral marginal bristles ; fifth with strong marginal bristles dorsally, displaced by abundant slender hairs below. Sternites short-haired. Fifth sternite as shown in fig. 10.

Wings. Costal spine wanting. Section III of costa slightly longer than section V. Wing membrane infuscated along the wing veins.

Legs. Mid-femur with comb ; mid-tibia with one antero-dorsal bristle. Hind femur and tibia conspicuously fringed on posterior side.

Terminalia. Genital segments rather small for the size of the body. First segment golden pollinose, without marginals. Second segment shining black. Morphological details as in fig. 9.

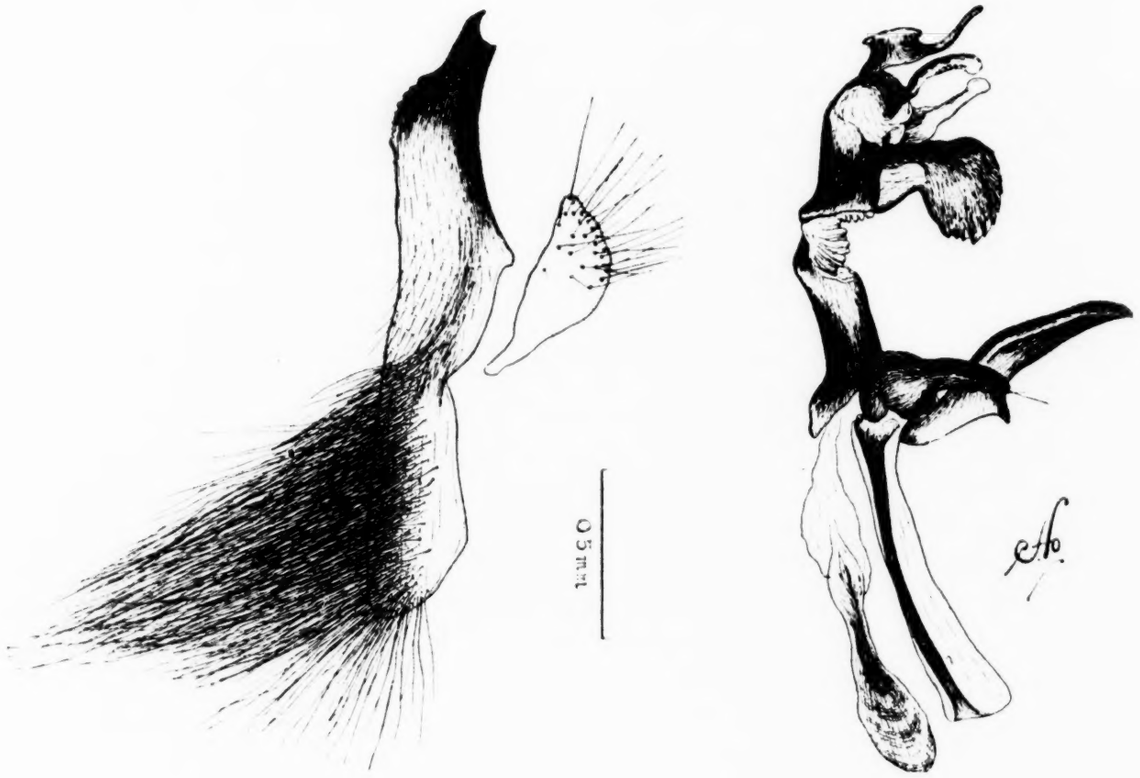


FIG. 9. *S. auricauda* sp. nov. Male terminalia.

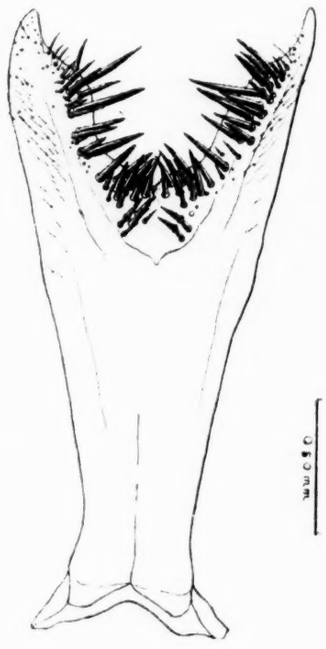


FIG. 10. *S. auricauda* sp. nov. Fifth sternite.

Length. 12·5–15·6 mm.

FEMALE. Unknown.

HOLOTYPE. Male, selected from a series of six males collected from W. Java, 600–800 m., Djampang, Tengah, G. Tjisoeroe, March, 1933 (M. E. Walsh).

PARATYPES. Five males, same locality and date.

On the morphology of the phallosome, this species is very closely allied to the preceding one, from which it may be separated by (1) the golden colour of the fourth and fifth abdominal segments, (2) its larger size, (3) its darker appearance, (4) the unfringed mid-femur, and (5) the various details of the structure of the male terminalia. The first of these distinguishing characters will also serve to separate it from all other species of the genus known to the writer.

Sarcophaga walshi sp. nov. (figs. 11, 12)

MALE. *Head.* Lateral verticals rudimentary. Frons 0·56 mm. wide in a single specimen. Frontal stripe with distinctly marked edges, slightly diverging below, at its narrowest part narrower than two parafrontalia taken together. Parafrontalia and parafacialia yellowish-silvery, the latter with a row of two or three longer hairs below, close to the eye. Frontals 11, the lowest three slightly diverging. Antennae black, third segment two and a half times the length of the second and reaching about three-fifths of the distance to the vibrissae, which are at the oral margin. Arista with long plumosity for three-fifths of its length. Vibrissal ridge with a few bristles above the vibrissal angle. Palpi black. Bucca about one-third the eye-height, with black hairs, none pale before the metacephalic suture. Back of head with two distinct and well-spaced rows of postocular cilia. Pale soft hairs around middle and below.

Thorax. Ground colour yellowish-grey, with the usual three black stripes and one fainter one on each side of the median stripe. Acrostichals only present as very weak prescutellars. Posterior dorsocentrals 5, the anterior three hardly differentiated. Scutellum with the usual bristles.

Abdomen. With the usual varying tessellation. Third segment with one pair of lateral marginal bristles. Fourth segment with one pair of median and three pairs of lateral marginal bristles. Fifth segment with the strong marginals displaced rather abruptly by finer ones on its ventral edges. Sternites short-haired. Fifth sternite as shown in fig. 12.

Wings. Costal spine distinctly present. Section III of costa equal to the length of section V. Wing membrane hyaline.

Legs. Mid-femur with comb ; mid-tibia with one antero-dorsal bristle. Hind femur and tibia without fringe.

Terminalia. First genital segment shining dark-brown on anterior half, posterior half grey pollinose, without marginals. Second genital segment shining black. Morphological details as in fig. 11.

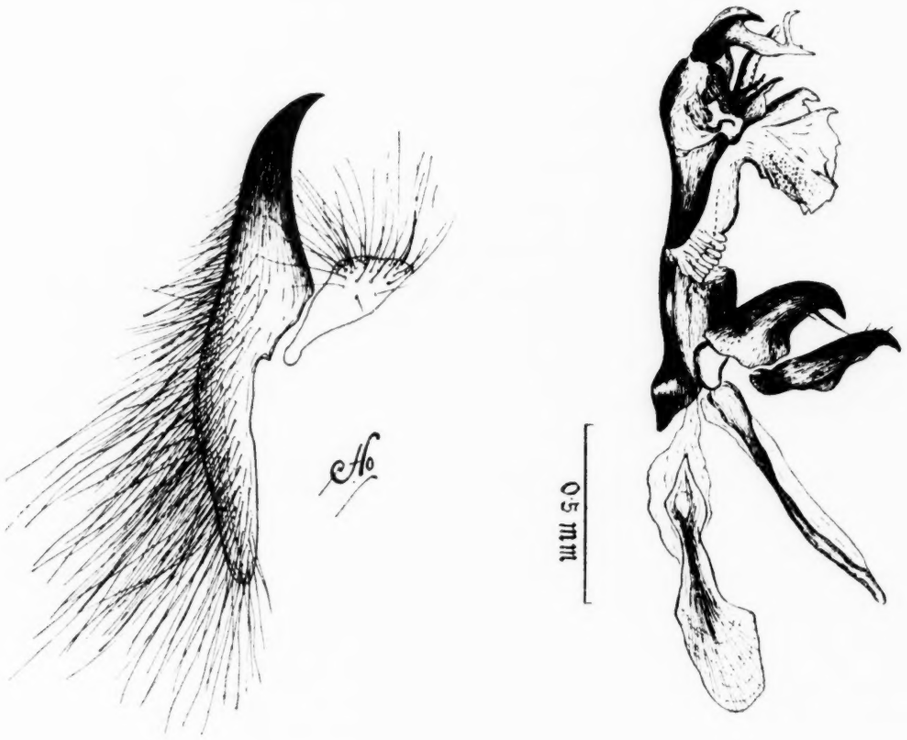


FIG. 11. *S. walshi* sp. nov. Male terminalia.

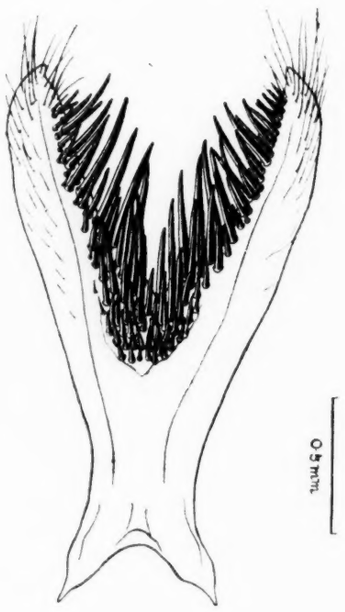


FIG. 12. *S. walshi* sp. nov. Fifth sternite.

Length. 11·0 mm.

FEMALE. Unknown.

HOLOTYPE. Male, a single specimen, W. Java, 600–800 m., Djampang, Tengah, G. Tjisoeroe, March, 1933 (M. E. Walsh).

Related to the Palaearctic species *S. similis* Pand. (Böttcher, 1913), from which it is separated by the absence of fringe on the femora and tibiae, and by the details of the structure of the terminalia.

REFERENCES

ALDRICH, J. M. (1932). New Diptera, or two-winged flies, from America, Asia, and Java, with additional notes. *Proc. U.S. Nat. Mus.*, **81**, art. 9, no. 2932, p. 15.

BÖTTCHER, G. (1913). Die männlichen Begattungswerkzeuge bei dem Genus *Sarcophaga* Meigen und ihre Bedeutung für die Abgrenzung der Arten. *Deutsch. Entom. Zeitschr.*, Hft. 1, p. 2, fig. 31.

HARDY, G. H. (1927). Notes on Australian and exotic Sarcophagid flies. *Proc. Linn. Soc. N.S.W.*, **52**, 447.

HO, CH'I (1932). Notes on Sarcophagid flies, with description of new species. I : *Albiceps*-group. *Bull. Fan Mem. Inst. Biol.*, **3**, 345.

PARKER, R. R. (1917). New flies of the genus *Sarcophaga* from Guam and the Philippines. *Proc. U.S. Nat. Mus.*, **54**, 89.

SENIOR-WHITE, R. (1924). A revision of the sub-family Sarcophaginae in the Oriental region. *Rec. Ind. Mus.*, **26**, 193.

A NOTE ON THE PATHOLOGY OF SCHISTOSOMIASIS DUE TO *S. JAPONICUM* AMONG CATTLE IN CHINA

BY

L. S. KAU

AND

KUANG WU

(*From the Division of Pathological Sciences, Henry Lester Institute of Medical Research, Shanghai*)

(*Received for publication April 8th*, 1938)

In another paper, Dr. Kuang Wu (1938) has recorded the results of a thorough investigation into the distribution in China of schistosome infection among cattle, which, as well as human beings, may act as hosts, harbouring the infection.

The study was made by securing at random portions of livers from the cattle slaughtered in the abattoirs of the International Settlement and the French Concession in Shanghai. In the laboratory these liver specimens were carefully identified, labelled and numbered. Portions were taken and minced into very fine pieces, put into glass flasks, and digested with potassium hydroxide 10 per cent. solution for about 8 days in an incubator at a temperature of 37° C.

At the end of the 8th day, the sediment was pipetted off and put on to a clean glass slide, where search for the ova of *Schistosoma japonicum* was made. Among the livers of 222 oxen and 213 water buffaloes examined to date, many have yielded positive findings for ova.

The work accomplished has revealed many interesting facts, which have led one of us (L. S. K.) to undertake a further study of the pathological lesions and changes noticed in these livers. In this investigation, frequent visits were made to the abattoirs of the two Settlements, in order to observe personally the gross specimens in their fresh condition.

The morbid anatomical changes noticed in these cattle were mainly in the spleen and the liver. Some of the spleens were enlarged, but the majority of them were obviously atrophic. The capsule was thickened, and adhesions to the adjacent viscera were observed on the surface and the hilum. The organ was red in colour and firm in consistency. On section, fibrosis of the capsule was noticed in places, giving an uneven thickness. The stroma also showed some fibrosis which stood out prominently in the firm and moist red pulp.

The livers were brown in colour and firm in consistency. The capsule was thickened and adhesions were observed mainly on the right lobe. The edges were sharp and regular. Most of these livers were small in size and much atrophied. Some presented a nodular appearance with an uneven surface,

chronic cirrhosis having taken place. Adhesions were also noticed on the concave surface, especially around the hilum, where the gall-bladder and the bile-ducts anastomosed. Sometimes fibrosis of the bile-ducts was evident, giving a cord-like feeling to the palpating hand. The gall-bladders were usually fibrous, with a firm and thickened wall. In the cavity some dark brown bile was present.

On section, the cut surface of the liver was moist and brownish in colour. The tissue was firm and the bile-ducts were cirrhotic, with the lumen reduced in size. In some, the lumen contained an organized embolus filling most of the space. Areas of congestion were noticed in places in the parenchyma of some of these livers. The connective tissue stroma was fibrotic, and a variegated appearance was produced in cases where the lobules were separated by these fibrous bands. In the atrophic livers there was an increased amount of connective tissue stroma. When calcification was present in the fibrous stroma, the knife cut with a gritty sound. In some cases *Fasciola hepatica* was found in the liver.

In some of the livers, small yellowish nodules, varying from the size of a pea to that of a cherry, were found on the surface. Some of these nodules were quite hard and calcified, and cut with a gritty sound. Others, when sectioned, looked like abscesses, the central portion being necrotic and the sloughs within greenish in colour. On occasion, these nodules, well encapsulated, were situated within the parenchyma of the liver, but the majority of them were found just beneath the capsular surface. So far no parasites have been encountered in the nodules.

In the case of the oxen, the liver and the spleen were often of normal size. There were a few patches of fibrosis on the surface, but the adhesions present were not as fibrotic as those usually observed in the livers of water buffaloes. The organ itself was firm and dark bluish in colour when *Fasciola hepatica* was present in numbers in the ducts ; the bile-ducts were fibrous in appearance, and the bile presented a dark brown colour. On section, the cut surface of the liver was moist, and dark red in colour. The bile-ducts and the parenchyma were often heavily bile-stained when *Fasciola hepatica* was present.

The spleens, red in colour, were not greatly enlarged. The capsule was not so thick as in the case of the water buffaloes, and few adhesions were found with the adjacent viscera.

Histologically, fibrosis was noticed in the connective tissue stroma and the capsule, indicating atrophic cirrhosis of the organ. There was chronic passive congestion present in the parenchyma. The biliary passages showed cirrhotic changes in the walls ; they were lined by a layer of tall columnar epithelial cells, which were even, regular and homogeneous in size and appearance. The liver-cell columns had undergone disintegration, and many red blood cells were found filling the sinuses. In the parenchyma and near the central vein, were scattered foci of round cell infiltration.

Some of these areas resembled the ' pseudo-tubercles ' usually described by other investigators. These ' pseudo-tubercles ' were found surrounded by a zone of round cells and a few eosinophiles ; necrosis of the central tissues had occurred. In the foci many endothelial cells were found surrounding the ova and forming a type of foreign-body giant cell. Owing to fixation the ova were distorted in shape and size ; many of them had undergone calcification ; their contents might or might not be visible. Eosinophilic cells were often found surrounding such an area. In certain of these areas, where no ova were present, there was evidence of necrosis of the tissues, and endothelial cells were found lying free.

Sometimes, when a single ovum was found lodged in the parenchyma of the ' pseudo-tubercle,' a radial arrangement occurred in the zone around the ovum. A few endothelial cells, some round cells and eosinophilic cells were usually present as well. When the ova were found lodged in the fibrotic stroma they were in most instances misshapen, crushed and calcified. No round cells were found infiltrating in such cases.

Occasionally, *Fasciola hepatica* was found lodged in the parenchyma vessels. Surrounding the worm there was some extravasation of blood in the adjacent soft tissues.

Sections taken from the subcapsular yellowish nodules histologically showed atrophy and fibrosis of the stroma. In the parenchyma were foci of necrosis of a miliary type. No ova were found in these lesions. In the sloughing necrotic area polymorphonuclear leucocytes, lymphocytes, and eosinophilic cells were present in numbers. The lesions appeared to be pyogenic in type, and were surrounded by a zone of round cells and few eosinophiles. The liver-cell columns also showed disintegration, and the nuclei were granular in appearance.

The present report is a preliminary study made on this work. Further investigations are still in progress, and we hope to give a fuller account of this problem in the near future.

ACKNOWLEDGEMENTS.—The authors wish to record their thanks to Dr. J. H. Jordan, Commissioner of the S.M.C. Public Health Department ; to Dr. J. Rabaute, Director of the French Municipal Public Health Department ; to Dr. R. C. Robertson, for the encouragement given in this investigation ; to Mr. R. V. Dent, for the photomicrographs reproduced ; and to Mr. A. J. Stonyer, for his technical assistance in the preparation of the histological sections.

REFERENCE

WU, K. (1938). Cattle as reservoir hosts of *Schistosoma japonicum* in China. *Amer. Jl. Hyg.*, **27**, 290.

EXPLANATION OF PLATE I

Fig. 1. Section of liver showing fibrosis of capsule and a pseudo-tubercle with ova beneath the capsule in the parenchyma. (× 97.)

Fig. 2. Section of liver showing the calcified ova in a pseudo-tubercle in the parenchyma. (× 97.)

Fig. 1

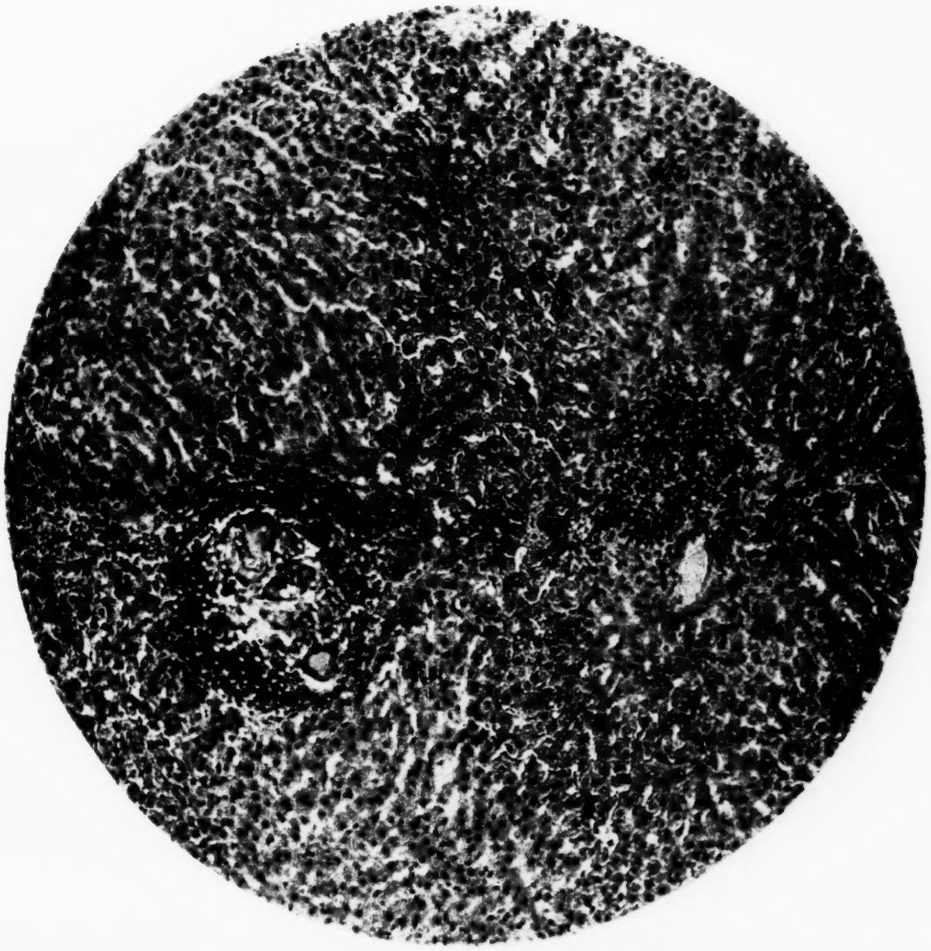


Fig. 2

UNIV. OF MICH.

H. R. Grubb, Ltd., Parker Walk, Crayden

PATHOLOGICAL FINDINGS AMONG PIGS EXPERIMENTALLY INFECTED WITH *FASCIOLOPSIS BUSKII*

BY

L. S. KAU

AND

KUANG WU

(*From the Division of Pathological Sciences, Henry Lester Institute of Medical Research, Shanghai*)

(*Received for publication April 8th*, 1938)

Observations have been made on the pathological lesions in pigs experimentally infected with the cysts of *Fasciolopsis buskii* during the past 18 months. The pigs were fed with cysts obtained from water caltrops collected in the vicinity of Hangchow. Studies were made to determine the incidence of these cysts in the several varieties of the water caltrops found. Pigs were used in this investigation because they have been found to be the most susceptible host.

Among the seven pigs autopsied in the laboratory, gross pathological changes were observed mainly in the gastro-intestinal tract—mostly in the duodenum, but also further down the tract in the jejunum.

The lesions consisted of minute foci of petechial haemorrhages scattered in the mucosal wall of the tract and level with the surface. At places where the worms were found attached to the mucosa there were patches of submucosal congestion. In some areas where the worms were detached from the wall, oedema was noted, and an imprint of the oral sucker could be seen under artificial light or with the aid of a hand-lens. No definite gross erosions of the mucosal surface could be seen. There was, however, a layer of slimy exudate on the surface. No parasitic ova were seen in smear preparations from the surface of the mucosal wall.

The serous surface of the intestinal tract showed no gross changes other than congestion of the vessels in places. The surface was smooth, moist and shiny. No thrombosis was noticed in the mesenteric vessels adjacent to the serous coat of the intestinal tract.

The heart and lungs showed passive congestion. The liver, spleen, pancreas and kidneys were also passively congested, but showed no further gross changes. The mesenteric lymph nodes were enlarged and congested.

Histologically, sections taken from the lesions of the duodenum showed oedema and congestion of the mucosa in places. There was extravasation of blood in the mucosa and the submucosa. In areas where the worms were found attached to the wall, the vessels were engorged with blood. The mucosal

PATHOLOGICAL FINDINGS AMONG PIGS EXPERIMENTALLY INFECTED WITH *FASCIOLOPSIS BUSKII*

BY

L. S. KAU

AND

KUANG WU

(*From the Division of Pathological Sciences, Henry Lester Institute of Medical Research, Shanghai*)

(*Received for publication April 8th*, 1938)

Observations have been made on the pathological lesions in pigs experimentally infected with the cysts of *Fasciolopsis buskii* during the past 18 months. The pigs were fed with cysts obtained from water caltrops collected in the vicinity of Hangchow. Studies were made to determine the incidence of these cysts in the several varieties of the water caltrops found. Pigs were used in this investigation because they have been found to be the most susceptible host.

Among the seven pigs autopsied in the laboratory, gross pathological changes were observed mainly in the gastro-intestinal tract—mostly in the duodenum, but also further down the tract in the jejunum.

The lesions consisted of minute foci of petechial haemorrhages scattered in the mucosal wall of the tract and level with the surface. At places where the worms were found attached to the mucosa there were patches of submucosal congestion. In some areas where the worms were detached from the wall, oedema was noted, and an imprint of the oral sucker could be seen under artificial light or with the aid of a hand-lens. No definite gross erosions of the mucosal surface could be seen. There was, however, a layer of slimy exudate on the surface. No parasitic ova were seen in smear preparations from the surface of the mucosal wall.

The serous surface of the intestinal tract showed no gross changes other than congestion of the vessels in places. The surface was smooth, moist and shiny. No thrombosis was noticed in the mesenteric vessels adjacent to the serous coat of the intestinal tract.

The heart and lungs showed passive congestion. The liver, spleen, pancreas and kidneys were also passively congested, but showed no further gross changes. The mesenteric lymph nodes were enlarged and congested.

Histologically, sections taken from the lesions of the duodenum showed oedema and congestion of the mucosa in places. There was extravasation of blood in the mucosa and the submucosa. In areas where the worms were found attached to the wall, the vessels were engorged with blood. The mucosal

glands were cystic in appearance and in a catarrhal condition. Some mucus exudate was present in the acini, and the columnar epithelial cells lining the wall were tall and proliferative. In the cytoplasm of some of these cells droplets of mucin were present ; in others some granules were observed, which looked like foreign bodies ingested by these cells.

On the surface, desquamated epithelial cells could be seen mixed with the mucus exudate, red blood cells, with various degenerated cells and debris from the tract. So far, no parasitic ova have been observed in the mucosal crypts or on the surface.

The vessels of the submucosal and the muscular layers were congested where the lesions were found. There were, however, no hypertrophic changes of the mucosal wall of the tract. The serous coat also showed no hyperplasia in the connective tissue.

The present note represents the observations so far made in this investigation. Further researches are in progress, and a more detailed account of the work will be given in the future.

ACKNOWLEDGEMENTS.—The authors wish to express their gratitude to Dr. R. C. Robertson, for the encouragement given in this investigation ; to Mr. R. V. Dent, for the microphotographs reproduced ; and to Mr. A. J. Stonyer, for his technical assistance in the preparation of the sections.

PLATE II

EXPLANATION OF PLATE II

Fig. 1. Section of the duodenum showing area of petechial haemorrhage in the mucosal wall. (× 97.)

Fig. 2. Section showing desquamation of the epithelial cells on the surface and the exudate in the lumen of the tract. (× 97.)

Fig. 1

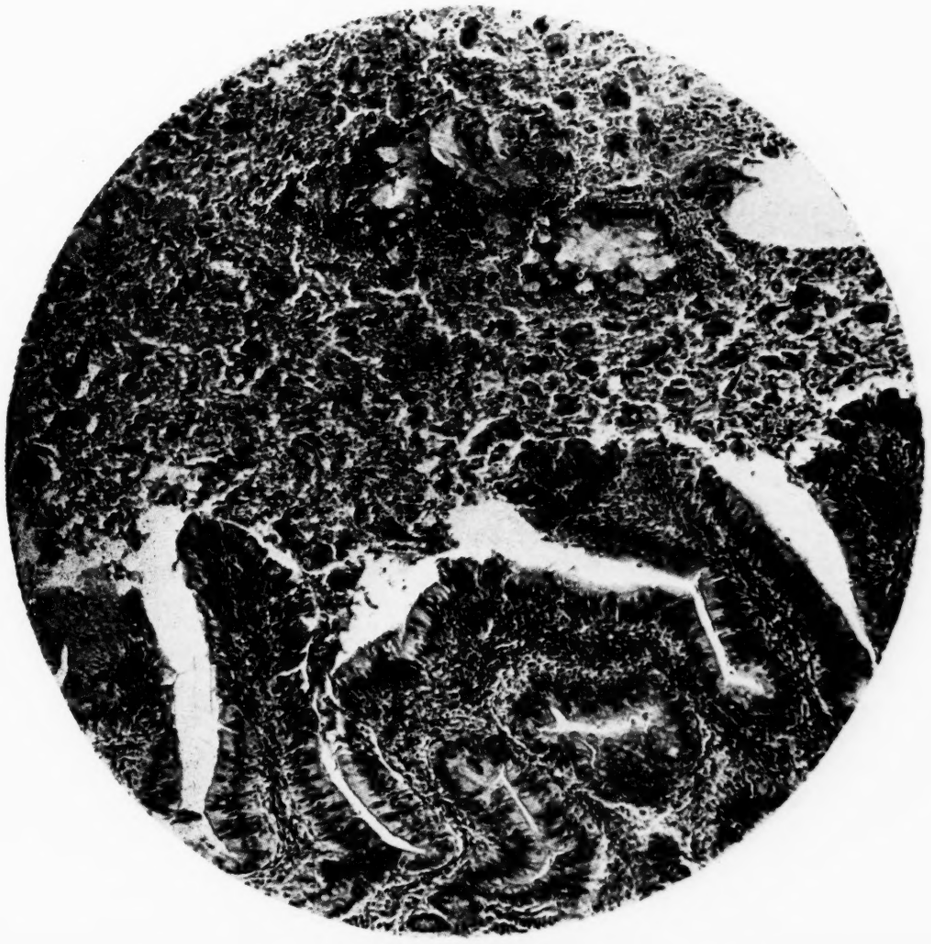


Fig. 2

H. R. Grubb, Ltd., Poplar Walk, Croydon

UNIV. OF MICH.

A CONTRIBUTION TO THE KNOWLEDGE OF THE SECOND INTERMEDIATE HOSTS OF *GNATHOSTOMA SPINIGERUM* OWEN, 1836

BY

SVASTI DAENGSVANG, Dr.P.H., D.T.M.

AND

PRADIT TANSURAT, M.B.

(*From the Department of Pathology, Faculty of Medicine, Chulalongkorn University, Bangkok, Siam*)

(*Received for publication April 25th*, 1938)

Prommas and Daengsvang (1933, 1936 and 1937) have written a series of reports on the life-cycle of *Gnathostoma spinigerum*, and have shown in their experiments that, after having been fed on infested cyclops (the first intermediate host), some species of fresh-water fish—namely, *Clarias batrachus* (Linnaeus) and *Ophiocephalus striatus* Bloch—act as the second intermediate hosts. The discovery of these experimental second intermediate hosts was at almost the same time confirmed by the work of Africa, Refuerzo and Garcia (1936), who found natural infestation by the parasite in three kinds of fresh-water fish in the Philippine Islands, namely, *Glossogobius giurus* Hamilton-Buchanan, *Ophiocephalus striatus* Bloch and *Therapon argenteus* Cuvier and Valenciennes. According to the above records, our information of the second intermediate hosts of the parasite was that they occurred only in some species of fresh-water fish. We feel that the findings of Chandler (1925) and of Africa and his co-workers (1936) in snakes are not to be regarded as conclusive, because the connection between the gnathostome larvae found in those snakes and the adult form of the parasite has not been clearly worked out. Further, although Prommas and Daengsvang experimentally found that some species of fresh-water fish are the second intermediate hosts of *Gnathostoma spinigerum*, none of the work in Siam had shown naturally infested fresh-water animals. We have therefore attempted to add to our previous knowledge of the problem.

MATERIALS AND METHODS

Forty *Ophiocephalus striatus*, 10 *Clarias batrachus* and 10 eels—*Monopterus albus* (Zuieuw)—were bought at random from different markets in Bangkok and Dhonburi. And 12 frogs (*Rana rugulosa* Wiegmann), which were kindly supplied by the Department of Physiology, were also bought from local dealers. These fresh-water fish and frogs are found abundantly everywhere in Siam, particularly in low-lying areas. They are used as food, and occasionally are eaten raw, both by human beings and by cats and dogs.

The animals were killed and dissected, and the flesh and viscera were thoroughly examined piece by piece, both with the naked eye and under the

microscope, after pressing between two glass slides. Encysted gnathostome larvae were found in all the types examined—mostly in muscle and liver, but also in the fat-bodies of the frog. After the encysted forms of the larvae in the animal tissue have once been seen, they will easily be recognized subsequently, even without the aid of a microscope.

The results obtained from our present examination are summarized in the following table.

TABLE

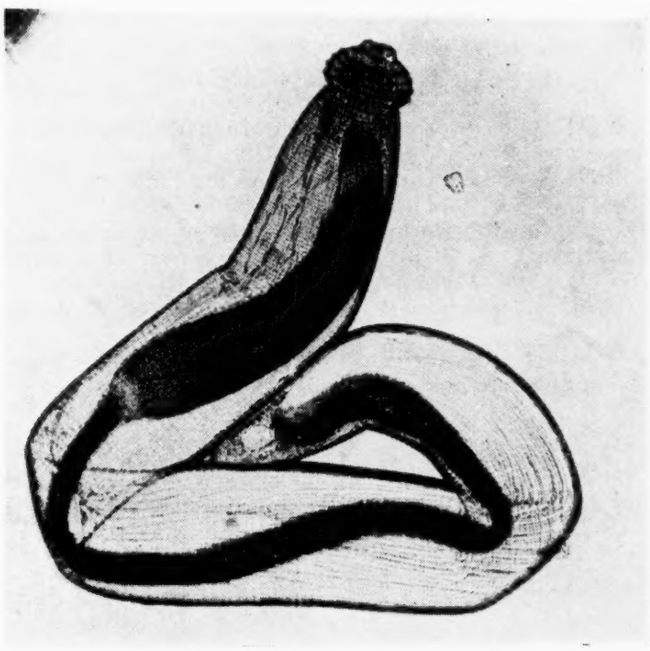
Showing the results of the examination of fresh-water fish and frogs for gnathostome larvae

Animal	No. examined	No. positive for larvae	Per cent. positive	Organs found infested	No. of larvae found	
					Min.	Max.
Rana rugulosa Wiegmann (frog)	12	11	91·67	muscle, liver and fat-body	2	83
Monopterus albus (Zuieuw) (eel)	10	8	80	muscle, liver	4	7
Ophiocephalus striatus Bloch	40	15	37·5	muscle, liver	1	6
Clarias batrachus (Linnaeus)	20	6	30	muscle	1	1

The naturally encysted gnathostome larvae thus found in frogs and fresh-water fish have practically the same structure and size as those found in the experimentally infested fish of Prommas and Daengsvang (1936, 1937) and in the naturally infested fish of Africa and his co-workers (1936). There is a definite cyst wall for each larva wherever it is found. The cyst is more-or-less rounded and is yellowish in colour, measuring 1·2 mm. in average diameter. The body of a larva after dissection from the cyst wall measures 4 mm. in length and 0·324 mm. in breadth. The cephalic bulb is 0·108 mm. from the anterior end to the cephalic constriction, and 0·216 mm. in transverse diameter. It has a pair of trilobed lips and is covered by 4 transverse rows of single-pointed spines. The cuticle is transversely striated throughout and covered with small single-pointed spines, which gradually become less in number and disappear at about the middle part of the body. Four cervical sacs are seen in the anterior portion. The alimentary canal consists of a strong muscular oesophagus and a well-developed intestine with subterminal anus. The columns of primary genital organs are seen near the intestine. (See microphotograph.)

DISCUSSION

In Siam, so far as we have worked out, some species of fresh-water fish—*Ophiocephalus striatus* Bloch and *Clarias batrachus* (Linnaeus)—eels—*Monopterus albus* (Zuieuw)—and frogs—*Rana rugulosa* Wiegmann—are found to be naturally infested with gnathostome larvae (presumably of *Gnathostoma spinigerum*). These animals are therefore evidently the second intermediate hosts of the parasite, and undoubtedly are among the chief sources of gnathostomiasis infestation for man and animals. The high percentage and heavy infestation by the parasite in frogs and eels in nature may be explained by the fact that these animals are known to travel great distances. We are still of the opinion that several other species of fresh-water fish, amphibians and reptiles may serve as the second intermediate hosts of *Gnathostoma spinigerum* in Siam. The search for additional second intermediate hosts of this parasite is now being continued in our laboratory.



Microphotograph of a *Gnathostoma spinigerum* larva after dissection from the cyst embedded in the muscle of a frog, *Rana rugulosa* Wiegmann.

SUMMARY

1. An attempt was made to find fresh-water animals naturally infested by *Gnathostoma spinigerum* larvae in Siam, and the method of the work is described.

2. Naturally encysted gnathostome larvae were discovered in 91·67 per cent. of frogs, *Rana rugulosa* Wiegmann ; in 80 per cent. of eels, *Monopterus albus* (Zuieuw) ; in 37·5 per cent. of *Ophiocephalus striatus* Bloch ; and in 30 per cent. of *Clarias batrachus* (Linnaeus).

microscope, after pressing between two glass slides. Encysted gnathostome larvae were found in all the types examined—mostly in muscle and liver, but also in the fat-bodies of the frog. After the encysted forms of the larvae in the animal tissue have once been seen, they will easily be recognized subsequently, even without the aid of a microscope.

The results obtained from our present examination are summarized in the following table.

TABLE

Showing the results of the examination of fresh-water fish and frogs for gnathostome larvae

Animal	No. examined	No. positive for larvae	Per cent. positive	Organs found infested	No. of larvae found	
					Min.	Max.
Rana rugulosa Wiegmann (frog)	12	11	91·67	muscle, liver and fat-body	2	83
Monopterus albus (Zuieuw) (eel)	10	8	80	muscle, liver	4	7
Ophiocephalus striatus Bloch	40	15	37·5	muscle, liver	1	6
Clarias batrachus (Linnaeus)	20	6	30	muscle	1	1

The naturally encysted gnathostome larvae thus found in frogs and fresh-water fish have practically the same structure and size as those found in the experimentally infested fish of Prommas and Daengsvang (1936, 1937) and in the naturally infested fish of Africa and his co-workers (1936). There is a definite cyst wall for each larva wherever it is found. The cyst is more-or-less rounded and is yellowish in colour, measuring 1·2 mm. in average diameter. The body of a larva after dissection from the cyst wall measures 4 mm. in length and 0·324 mm. in breadth. The cephalic bulb is 0·108 mm. from the anterior end to the cephalic constriction, and 0·216 mm. in transverse diameter. It has a pair of trilobed lips and is covered by 4 transverse rows of single-pointed spines. The cuticle is transversely striated throughout and covered with small single-pointed spines, which gradually become less in number and disappear at about the middle part of the body. Four cervical sacs are seen in the anterior portion. The alimentary canal consists of a strong muscular oesophagus and a well-developed intestine with subterminal anus. The columns of primary genital organs are seen near the intestine. (See microphotograph.)

DISCUSSION

In Siam, so far as we have worked out, some species of fresh-water fish—*Ophiocephalus striatus* Bloch and *Clarias batrachus* (Linnaeus)—eels—*Monopterus albus* (Zuieuw)—and frogs—*Rana rugulosa* Wiegmann—are found to be naturally infested with gnathostome larvae (presumably of *Gnathostoma spinigerum*). These animals are therefore evidently the second intermediate hosts of the parasite, and undoubtedly are among the chief sources of gnathostomiasis infestation for man and animals. The high percentage and heavy infestation by the parasite in frogs and eels in nature may be explained by the fact that these animals are known to travel great distances. We are still of the opinion that several other species of fresh-water fish, amphibians and reptiles may serve as the second intermediate hosts of *Gnathostoma spinigerum* in Siam. The search for additional second intermediate hosts of this parasite is now being continued in our laboratory.

Microphotograph of a *Gnathostoma spinigerum* larva after dissection from the cyst embedded in the muscle of a frog, *Rana rugulosa* Wiegmann.

SUMMARY

1. An attempt was made to find fresh-water animals naturally infested by *Gnathostoma spinigerum* larvae in Siam, and the method of the work is described.

2. Naturally encysted gnathostome larvae were discovered in 91·67 per cent. of frogs, *Rana rugulosa* Wiegmann ; in 80 per cent. of eels, *Monopterus albus* (Zuieuw) ; in 37·5 per cent. of *Ophiocephalus striatus* Bloch ; and in 30 per cent. of *Clarias batrachus* (Linnaeus).

3. Encysted gnathostome larvae were found mostly in the muscle and liver, but were also found in the fat-bodies of the frog.

4. The morphology of the larva is described.

5. At present the second intermediate hosts of *Gnathostoma spinigerum* may be summarized to include some species of fresh-water fish, frogs and probably snakes.

6. The search for additional second intermediate hosts of this parasite is in progress.

ACKNOWLEDGEMENTS.—We wish to acknowledge with thanks the valuable advice and helpful criticism of Dr. Chalerm Prommas, Head of the Department of Pathology. We are also indebted to Dr. Luang Lipidharm, Head of the Department of Physiology, for the supply of frogs, and to Luang Chula, of the Bureau of Fisheries, for the identification of frogs and eels. We wish also to thank Dr. Khun Ketudasana, of the Department of Pathology, for micro-photography.

REFERENCES

AFRICA, C. M., REFUERZO, P. G., and GARCIA, E. Y. (1936*a*). Observations on the life cycle of *Gnathostoma spinigerum*. *Philipp. Jl. Sci.*, **59**, 513.

——— ——— ——— (1936*b*). Further observations on the life cycle of *Gnathostoma spinigerum*. *Ibid.*, **61**, 221.

CHANDLER, A. C. (1925). A contribution to the life-history of a gnathostome. *Parasitol.*, **17**, 237.

PROMMAS, C., and DAENGSVANG, S. (1933). Preliminary report of a study on the life-cycle of *Gnathostoma spinigerum*. *Jl. Parasitol.*, **19**, 287.

——— ——— (1936). Further report of a study on the life cycle of *Gnathostoma spinigerum*. *Ibid.*, **22**, 180.

——— ——— (1937). Feeding experiments on cats with *Gnathostoma spinigerum* larvae obtained from the second intermediate host. *Ibid.*, **23**, 115.

THE STUDY OF THE MALE AND FEMALE TERMINALIA OF THE GENUS *SARCOPHAGA*, WITH ILLUSTRATIONS OF THE TERMINALIA OF THE *HAEMORRHOIDALIS* GROUP

BY

W. S. PATTON

AND

CH'I HO

(*From the Department of Entomology, Liverpool School of Tropical Medicine*)

(*Received for publication May 3rd*, 1938)

INTRODUCTION

The genus *Sarcophaga* at present contains some 650 species from all the zoological regions of the world. As is well known, all the species are larviparous in habit, depositing first stage larvae. Many species are domestic insects and scavengers, breeding in decaying animal matter and excreta of all kinds; others again are parasites of insects—chiefly of grasshoppers and locusts, and probably of other insects as well, for one of us (W.S.P.) recently found three larvae of some unknown species in the abdomen of a male *Sarcophaga brevicornis* Ho from Kuala Lumpur; that the larvae were parasites is quite clear from the fact that one was still in the first stage while the other two were second stage larvae. The females of the parasitic forms usually have relatively few first stage larvae, while the scavengers often have more, though the number is never very great.

TAXONOMIC STUDY OF THE SPECIES

The taxonomic study of the species of *Sarcophaga* may be divided into three periods, as follows:

First Period. 1826–1911. The first period dates from 1826, when Meigen established the genus and described 30 species. He gave a full and tolerably accurate account of the male terminalia of the species then known to him, but without any illustrations. The next important contribution in this period was that of Pandellé (1896), who also described the terminalia of many species, but again without any illustrations.

Second Period. 1911–1934. The second period may be said to have begun with Kramer's paper, 'Die Tachiniden der Oberlausitz,' in 1911, in which he illustrated in outline the terminalia of a number of European species. This was followed by Böttcher's remarkable papers (1912-13), in which he used the characters of the male terminalia to great effect in separating closely allied species, and, more important still, in finally settling the identity of many of

Meigen's and Pandellé's species. His simple outline drawings have been most helpful in the identification of the species. Since then, remarkable progress has been made in this period, not only in elucidating the species of the older authors, but also in adding to our knowledge of the world's species of the genus. Special mention must be made of the works of Parker (1916–1923), who, we believe, was the first to group species solely on the characters of the male terminalia. Monographic studies of the species of the different faunistic areas were rapidly published, and in each case the male terminalia have been illustrated, thus rendering identification easy and certain. The pioneer work of Aldrich on the North American species calls for special mention, as well as Hall's excellent studies, especially of the Central American species ; for the Palaearctic fauna we have the work of Villeneuve, Rodendorf, Baranof, Séguy, Wainwright and others ; for the Oriental species we have the monograph of Senior-White ; Curran has recently studied some of the Ethiopian species ; Salem has worked on the Egyptian species ; the Chinese species have been studied by Ch'i Ho ; and, lastly, the Australian species have been studied by Hardy and Johnston. Many other authors have described species from the different faunas.

Third Period. 1934– The third period began in 1934, when one of the writers (W.S.P.) for the first time used a new technique in the study of the terminalia. Previous to this date, one of two methods was employed in the study of the male terminalia : (1) The terminalia were drawn out—or spread, to use the more common term in use—and left attached to the specimen ; this exposed the anal cerci, the distal segment of the ninth coxite, and most of the phallosome and parameres. (2) After the terminalia had been spread, they were removed with needles and mounted in a drop of Canada balsam or other suitable mounting medium on a card with the specimen, or on a piece of celluloid, so as to show the parts in side view. A drawing of the entire terminalia was then executed, and these drawings show the tenth tergum with the attached anal cerci, the distal segment of the ninth coxite, and the phallosome and parameres projecting from beneath the cerci ; in most of these drawings the paramere is usually incompletely illustrated. Owing to the fact that the structure of the phallosome and of the anal cerci, as seen in side view, are absolutely distinctive and characteristic of each species, it is relatively easy to identify any species from such drawings (even when in outline). In some of these drawings the details are, however, not good, owing not only to the small size of the drawings, but to the fact that it is difficult from the nature of the mounts accurately to depict the parts (particularly the various gadgets of the phallosome). And, indeed, in the case of closely allied species it is sometimes impossible to be sure of the species described.

In the new technique for the study of the terminalia noted above, either the entire abdomen is removed, or only the end is cut off to include all the terminalia, especially sternum 5 (which, it should be noted, is not, strictly

speaking, part of the terminalia). The abdomen, or part of it, is next placed in caustic potash until sufficiently cleared, and is then taken through the alcohols to clove oil. At this stage the fifth sternum is removed and the relations of the parts are studied *in situ*, so as to become familiar with their relationships. Next, the ninth tergo-sternum is dissected off from the tenth tergum, great care being taken not to damage it. The next step in the dissection is the most difficult, viz., dissecting off the phallosome from the ninth tergo-sternum so as to avoid damaging the tergo-sternum and loosening the two parts of the paramere. The fifth sternum is now dissected off, and this, with the phallosome (and parameres attached) and the ninth tergo-sternum, are placed in a small drop of liquid Canada balsam on a slide, and orientated as the observer wishes, the phallosome being placed on its side. When fixed, it is covered with fresh balsam. In another large drop on the same slide the tenth segment with the anal cerci and the distal segment of the ninth coxite are placed so that the whole may be seen in side view. The parts can then be illustrated in detail, and accurate drawings of the structure of the phallosome with one paramere, the ninth tergo-sternum and the fifth sternum can be executed; similarly the tenth tergum with attached anal cerci can be illustrated in side view. Later the tenth tergum can be removed from the slide and returned to the clove oil, and the anal cerci and the distal segments of the ninth coxite can be dissected off, mounted in ventral view, and then drawn. It should particularly be noted that at no stage in this technique is a cover-slip used.

This method of studying the male terminalia provides not only permanent specimens, but the means of making accurate drawings of the parts, so as to facilitate comparative studies.

The next advance in the study of the terminalia which was initiated in 1934 was the study of the female parts, by dissecting them off and mounting them on a slide, after clearing in caustic potash. Up to this date, it has been admitted by all students of the genus that it is exceedingly difficult to identify with certainty the females on external characters alone; in most keys to the species it is definitely stated that they are only of use for the male sex. There are possibly some students of the group who may not agree with this statement, and who may say that they can identify many of the females apart from the males. In reply, we can only say that a few females can undoubtedly be identified with reasonable certainty on external characters, but that, in the case of the majority, there is always such a large margin of doubt that one cannot be sure of the species. This, of course, refers to females caught quite apart from males. The study of the female terminalia, however, opens up an entirely new field of taxonomic work, which will, when the parts have been illustrated—as has been done in the case of the males—enable anyone to identify this sex with certainty. In a series of papers which are now being published by Patton and Wainwright

(1935–37) the male and female terminalia of the species of *Sarcophaga* of the British Isles are being illustrated, and the characters of these structures are correlated with the more easily studied external characters. This is the first time that such a study has been undertaken. The study of the female terminalia of the species of this fauna has clearly demonstrated the fact that they afford characters of specific value.

SPREADING THE MALE TERMINALIA

By the term ' spreading ' is understood the drawing back of the anal cerci so as to expose them as well as the phallosome and other parts which are concealed in the genital atrium in the position of rest. It will be remembered that when not in use the anal cerci are bent forward above the fifth sternum between its two forks or lateral arms, and are thus concealed ; if left in this position, they harden, so that later they cannot be drawn back satisfactorily, even after relaxing the specimen ; and when trying to do so one is apt to damage the parts. It is best, therefore, to spread the parts soon after catching the specimen. The following technique has been devised by one of the writers (Ch'i Ho) and has proved most satisfactory, as the parts are then permanently displayed.

The method is essentially one that has been described in Senior-White's paper (1924) but with some important modifications. After killing, the specimen is left over a period of about six hours for complete relaxation. The length of time necessary for this varies considerably with the weather conditions as well as with the size of the specimens. It is well known that when an insect dies it passes through a period of post-mortem rigidity, followed by a period of relaxation, after which it again becomes hardened. To carry out the ' spreading ' at a well-chosen time not only facilitates the operation, but also greatly increases its speed, which latter is of importance when a large number of specimens have to be dealt with. A period of about six hours was found quite sufficient for relaxation in most cases under summer conditions in China.

The specimen is then pinned with a strip of paper immediately beneath it. A number of such strips were cut ready for use before the pinning and spreading were begun. They are of various sizes to suit different specimens—their lengths should slightly exceed those of the specimens and their widths should be about 1 cm. These strips, besides providing a means to prevent the subsequent contraction of the terminalia after having been drawn out, are also used as a stage on which the out-spreading legs rest, which latter condition is also desirable for study. Ordinary type-writing paper of better quality was found good for this purpose.

The terminalia are extracted by means of a pair of fine forceps, or, perhaps better, by inserting a needle-point into the space immediately before the anal cerci and drawing the terminalia posterior-wards. A familiarity with the position of the terminalia in repose can easily reveal the whereabouts of the space. If a specimen has been properly relaxed, a little force is usually sufficient to draw

the terminalia backwards ; and the anal cerci of the genus are of such strength that no damage should be done by the little force exercised. If, however, any difficulty is encountered, that must be due to insufficient relaxation ; the best thing to do then is to lay down the specimen for another couple of hours rather than to proceed with the operation, which will in the end only prove damaging to the parts.

No matter how well the specimens have been relaxed, the terminalia usually pull back again as soon as the traction force is released ; and if in some cases they remain extracted they are often not in the desired position for use. To keep the organ in a properly exposed condition, Aldrich (1916, p. 15) and Johnston and Hardy (1923, p. 98) adopted the cork and secondary pin method ; Senior-White adopted a method of nicking the edges of the membranous lateral margin of the first genital segment. It will, however, be found much simpler and quicker to use the strip method instead. Drawing the terminalia as far back as they will go, the strip is raised to such a position that its posterior edge just meets and pushes against the apices of the anal cerci. If the strip proves too long, cut off a little bit ; otherwise use a longer one. A strip of better-quality type-writing paper will be found of sufficient strength to sustain the retraction force of the anal cerci. When the terminalia are thus fully extended, the phallosome must also fall into position ; if it remains concealed it is necessary to insert the point of a needle in front and above and lever it out. After leaving the specimen to dry for two or three days, remove the strip by cutting it into pieces. The specimens so treated will keep in good order in regard to both terminalia and legs.

MECHANISM OF COPULATION

It is well known that species of *Sarcophaga* taken *in cop.* are of great value, for then one is certain of the female. Such pairs are usually separated, or they separate themselves, after they have been caught and placed in the killing-bottle ; they are then usually pinned together on the same pin. The mechanism of copulation has, therefore, been little studied, if at all. One of the writers has made some preliminary observations which have led to the following conclusions.

There are apparently three possible mechanisms of copulation in *Sarcophaga*, as follows :

1. The anal cerci are only used to grasp the end of the female terminalia externally in a dorso-ventral position. The extension of the cerci opens the male genital atrium, into which the end of the female terminalia passes, and the phallosome then enters the genital opening. It is probable that this is the mechanism of copulation in those species which separate as soon as they are caught in a net or a tube, before they are transferred to the killing-bottle.

2. The anal cerci, after extension, are inserted into the genital opening and flexed backwards, thus drawing back the dorsal end of the female terminalia ; the phallosome then passes between the anal cerci, when it is erected and enters

the genital opening. This peculiar mechanism was observed in the case of *Sarcophaga rosellei* (Patton and Wainwright, 1935, p. 522, fig. 5). Two pairs were caught *in cop.*, and in both cases they remained fixed after being caught and transferred to a killing-bottle. The abdomens were cut off and placed in caustic potash, and even with softening of the parts the anal cerci remained fixed in position. It was noted that the chitinous plate on the dorsal wall of the vagina (signum) was bent back, and that

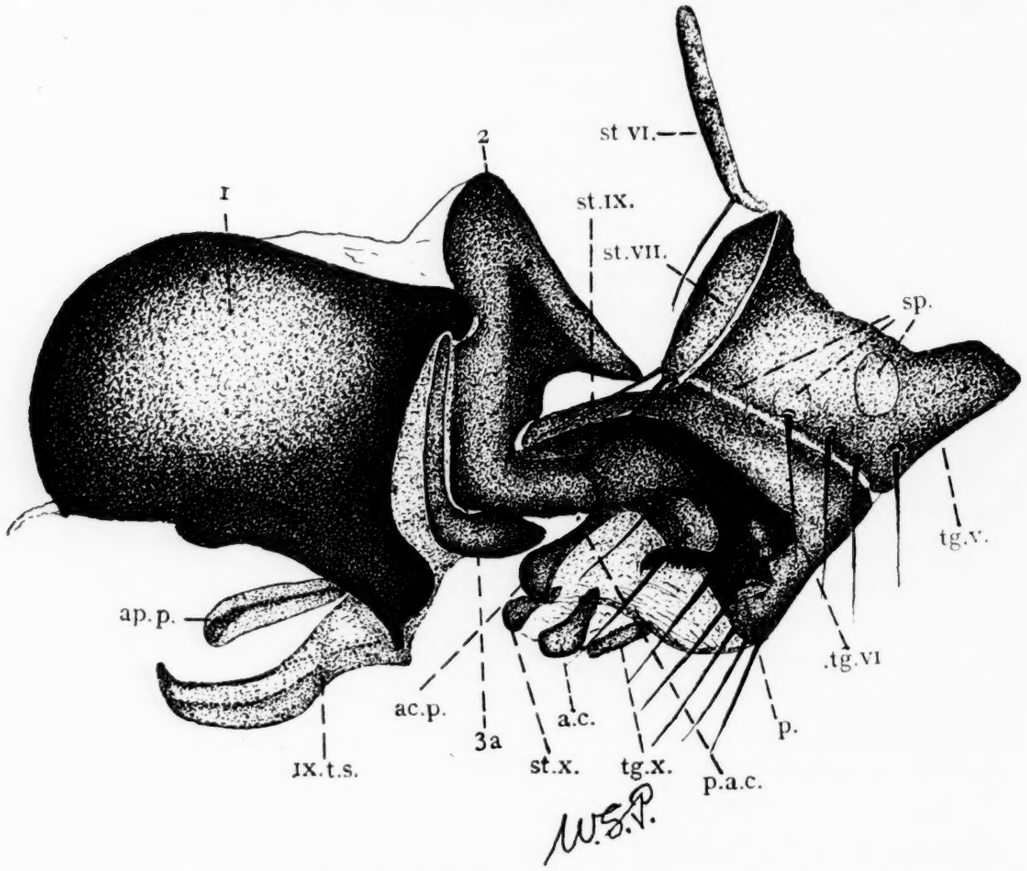


FIG. 1. Ends of abdomen of ♂ and ♀ *S. robusta*, to show mechanism of copulation ; the male parts have been drawn into line with those of the female, in order to facilitate the illustration—normally they are on the dorsal side of the female. *a.c.*—Anal cercus of female ; *ac.p.*—Accessory plate of female ; *ap.p.*—Apodeme of phallosome ; *p.*—Phallosome ; *p.a.c.*—Process of anal cercus of male ; *sp.*—Spermathecae ; *st.vi*, *st.vii*, *st.ix*, *st.x.*—Sixth, seventh, ninth and tenth sterna ; *tg.v*, *tg.vi*, *tg.x.*—Fifth, sixth, tenth terga ; 1.—Tenth tergum of male ; 2.—Anal cercus ; 3*a*.—Distal segment of ninth coxite ; *ix.t.s.*—Ninth tergo-sternum.

it appeared to act as a support to the wall and to prevent its being injured by the relatively sharp cerci of the male. We do not know how common this mechanism is in the genus, nor whether it is associated with a particular type of signum.

3. The third mechanism is illustrated in fig. 1, which shows the relations of the parts during the act of copulation in *Sarcophaga robusta*. The pair was sent to one of us by Mr. Dormer from California. The male was pinned, and

the rather small female was dangling from the end of the abdomen. *Sarcophaga robusta* in the male sex is of peculiar interest, for, as far as we know, it is the only species which has a large dorso-lateral accessory process connected with each

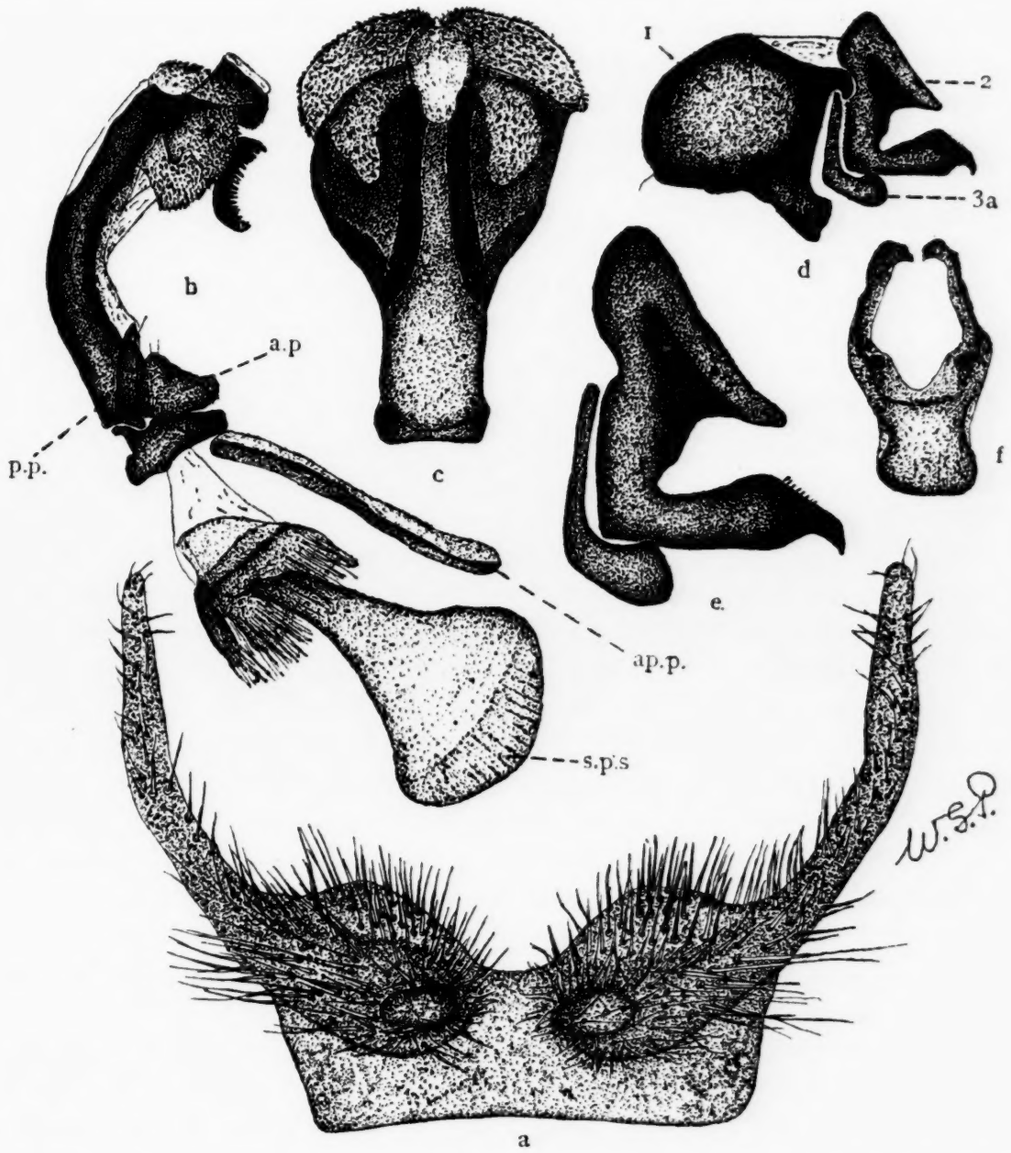


FIG. 2. *a.*—Fifth sternum of *S. robusta*; *b.*—Phallosome and one paramere in side view; *a.p.*—Anterior paramere; *ap.p.*—Apodeme of phallosome; *p.p.*—Posterior part of paramere; *s.p.s.*—Sperm pump sclerite; *c.*—Ventral view of phallosome; *d.*—Lateral view of tenth tergum, to show anal cercus and distal segment of ninth coxite; lettering as in fig. 1; *e.*—Lateral view of anal cercus, to show the accessory process and the distal segment of the ninth coxite; *f.*—Ninth tergo-sternum.

anal cercus. According to Aldrich (1916) it is widely distributed in the United States of America and has been recorded from Cuba; it apparently breeds in carcases of animals and exposed beef.

Without studying the mechanism of copulation and the structure of the parts, it would be impossible to understand the use of the accessory process of the anal cercus. A reference to fig. 1, which is drawn from the specimen noted, will show that the accessory processes are passed into the genital opening on each side of the phallosome, and that the blunt point of the small fused anal cerci is merely pressed against the ventral wall of sternum 9. The distal segment of the ninth coxite is a long narrow plate expanded at the end, which is bent inwards ; it clearly takes no part in clasping the end of the larvipositor, but its structure suggests that it may have been a clasper at one time, or at least that this species had descended from a far-distant ancestor in which the distal segment was a clasper. It seems obvious that the function of the pair of accessory hooks of the anal cerci is to expand the genital opening laterally, and this view receives the support when the structure of the phallosome is studied in dorso-ventral view. The phallosome is illustrated in fig. 2, *b*, in lateral view, and in fig. 2, *c*, the end is shown in ventral view. It will be noted that in lateral view the phallosome is relatively narrow, the main body being very short and wide and hinged to the long, strongly chitinized, distal part, which ends in a wide oval opening (of ejaculatory duct). At each side of the distal end there is a large, semi-chitinous, heavily spined flap, and projecting from the dorsal surface on each side there is a strongly chitinized heavily spined process which has a characteristic appearance in side view (fig. 2, *b*). The paramere is very small (fig. 2, *b*), the posterior part being a small raised plate with a hair near the end, typical of this part in most species of *Sarcophaga* ; the anterior part is a small triangular plate. In the position of copulation, the parameres do not enter the genital opening, and in fig. 1 they are lying just behind the expanded end of the distal segment of the ninth coxite, and are obscured. It will be noted, then, that, although the phallosome appears to be a narrow chitinous tube in side view, the distal end is very wide—as wide as the genital opening. It seems, therefore, very reasonable to assume that the accessory processes of the anal cerci are used to expand the opening laterally, in order to facilitate the entrance and to guide the phallosome.

The female terminalia (fig. 3) are also of peculiar interest in that sterna 7 and 9 are wide rounded plates which overlap tergum 6, which is a single plate ; in many species of *Sarcophaga* this plate is divided dorsally into two parts. In addition, there is a wide, strongly chitinized, accessory plate attached to the distal end of sternum 10, which in the position of copulation overlaps it ; it would appear that this plate supports and strengthens the dorsal wall of the genital opening. There is no signum.

The study of the mechanism of copulation in *S. robusta*, then, clearly explains the function of the accessory processes of the anal cerci, and, in the female, the function of the large accessory plate attached to the tenth sternum. We cannot help feeling that *S. robusta* is a primitive species and perhaps the only existing link with some past ancestor. That this may be the case is suggested

by the small fused anal cerci, which are quite unlike those of any present-day species known to us. The distal segment of the ninth coxite is suggestive of some more efficient lateral clasper, which was possessed by a long-vanished ancestor when the female terminalia were longer. The phallosome, too, is relatively simple for a *Sarcophaga*, in that it possesses relatively few of the gadgets which are so common on the phallosome of most species. This third method of copulation, in which an accessory process has developed in connection with the anal cercus, for the purpose of expanding as well as of holding the

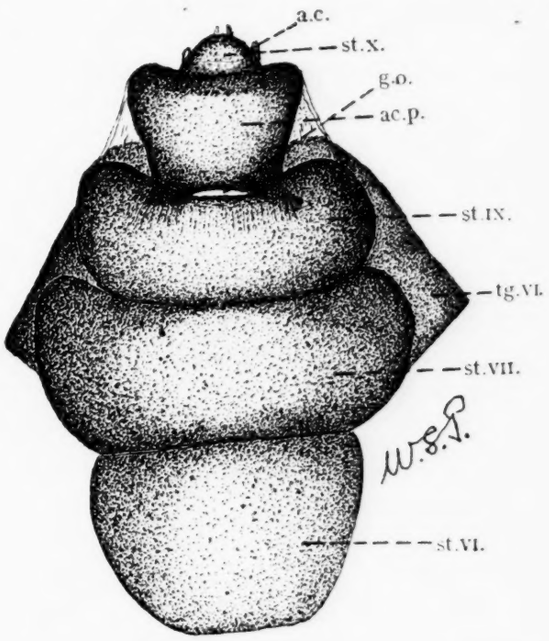


FIG. 3. Ventral view of terminalia of ♀ *S. robusta* ; *g.o.*—Genital opening ; other lettering as in fig. 1 ; note the large convex accessory plate ; study this illustration with that in fig. 1.

female abdomen, is probably unique in the genus. The other two methods noted are probably the common mechanisms in use.

GROUPS OF *SARCOPHAGA* BASED ON TERMINALIC CHARACTERS

We believe that Parker was the first to appreciate the grouping of the species solely on the structure of the male terminalia ; since then, others have followed up his suggestions, and we now know of at least two well-established groups, the *misera* and the *albiceps* groups. One of the writers (Ch'i Ho) has recently recorded his study of the latter. We would regard these as primary groups. There are others which have yet to be elucidated, and in these there are smaller or secondary groups of species which, though exhibiting the fundamental structural characters of the terminalia of the primary groups, possess others which associate them more closely with some of the species than with others of the primary group. And here we should like to point out that these secondary

groups can only satisfactorily be elucidated by employing the new technique noted above—that is, by dissecting the parts after they are cleared and by studying with high powers of the microscope, without compression. With such preparations it is possible to make a complete and exact study of the phallosome, for instance.

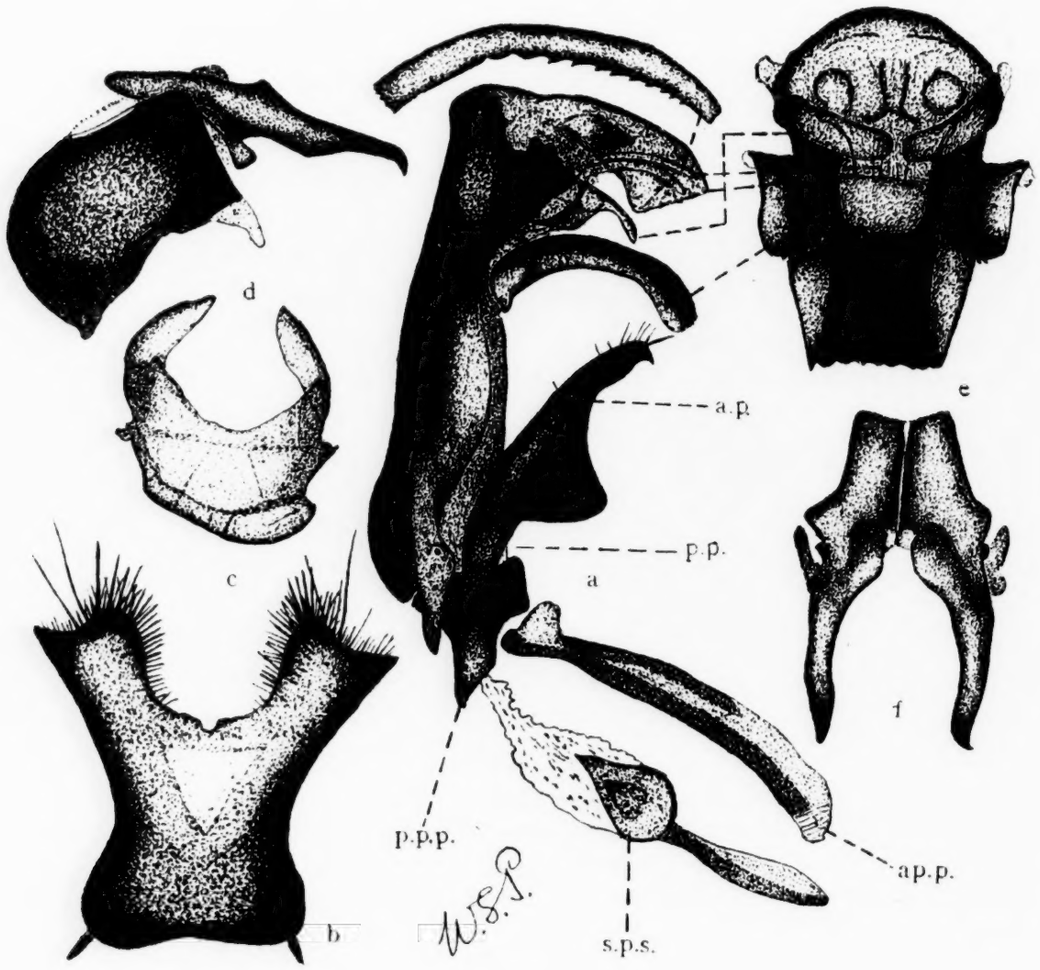


FIG. 4. *a.*—Phallosome and one paramere of *haemorrhoidalis* in side view; *p.p.p.*—Posterior process of phallosome; other lettering as in fig. 2, *b*; *b.*—Fifth sternum; *c.*—Ninth tergo-sternum; *d.*—Tenth tergum, anal cercus and distal segment of ninth coxite in side view; *e.*—Dorsal view of end of phallosome; *f.*—Ventral view of anal cerci and distal segments of ninth coxites.

We now propose to give some notes on a small group of species which has, as far as we know, not been recognized.

HAEMORRHOIDALIS GROUP

In a recent paper, Patton and Wainwright (1937) have illustrated and noted the salient diagnostic characters of the terminalia of *S. haemorrhoidalis*; the illustrations, together with notes, are reproduced here.

Sarcophaga haemorrhoidalis Meigen. SALIENT DIAGNOSTIC CHARACTERS OF TERMINALIA. MALE. Red. The anal cercus, when seen in side view (fig. 4, *d*), is a long narrow plate with a characteristic hump on the ventral side about the middle; at the point where the two cerci are joined there is another; the free end of the cercus narrows, and is bent upwards and shaped like a beak (fig. 4, *d*). In ventral view (fig. 4, *f*) it will be noted that about three-quarters of each cercus is free, the remainder being joined to its fellow. The distal segment of the ninth coxite is long and narrow, and the free end

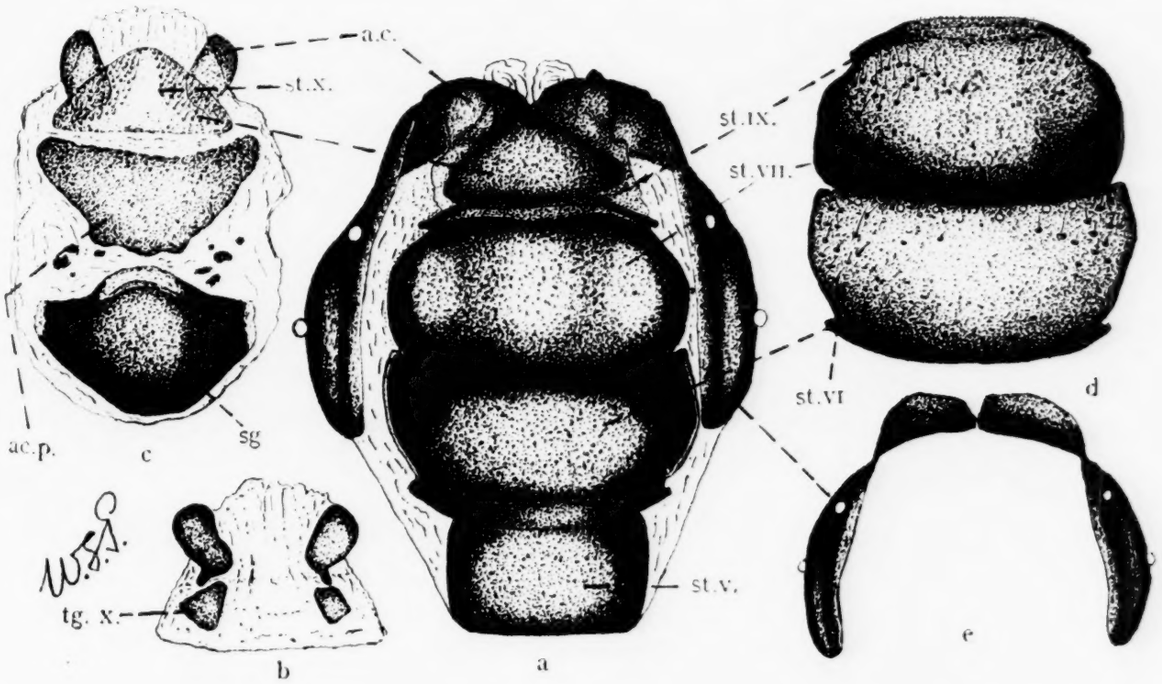


FIG. 5. *a.*—Ventral view of terminalia of ♀ *haemorrhoidalis*, to show diagnostic characters; *st.v.*—Fifth sternum; other lettering as in fig. 1; *b.*—Dorsal view of end of terminalia, to show anal cerci and tenth tergum in two parts; *c.*—Ventral view of end of terminalia, to show anal cerci, tenth sternum, accessory plate and signum (*sg.*); *d.*—Sixth, seventh and ninth sterna; *e.*—Sixth tergum, to show that it consists of two plates; note dorsal notch.

is dilated. The phallosome is long, consisting of the usual two parts; the proximal is very short, ending ventrally in a broad but short posterior process which, when seen in side view, ends in a short blunt point; the distal part is a long, wide, chitinous tube ending distally on the ventral side in a broad semi-chitinous hood. On each side of the ventral surface, nearer the distal than the proximal end, there is a stout chitinous plate bent inwards, the rounded end finely serrated; distal to this process and on each side of the middle of the ventral surface there is another broad chitinous plate directed towards its fellow (fig. 4, *a*, *e*); when seen in side view, this plate appears as a narrow finger-like

process, owing to its being bent so that its true structure can only be made out when the phallosome is examined from the ventral side. Projecting from under the terminal hood-like end of the phallosome, there is a pair of long serrated rods, one of which is shown enlarged in fig. 4, *a*. The anterior paramere

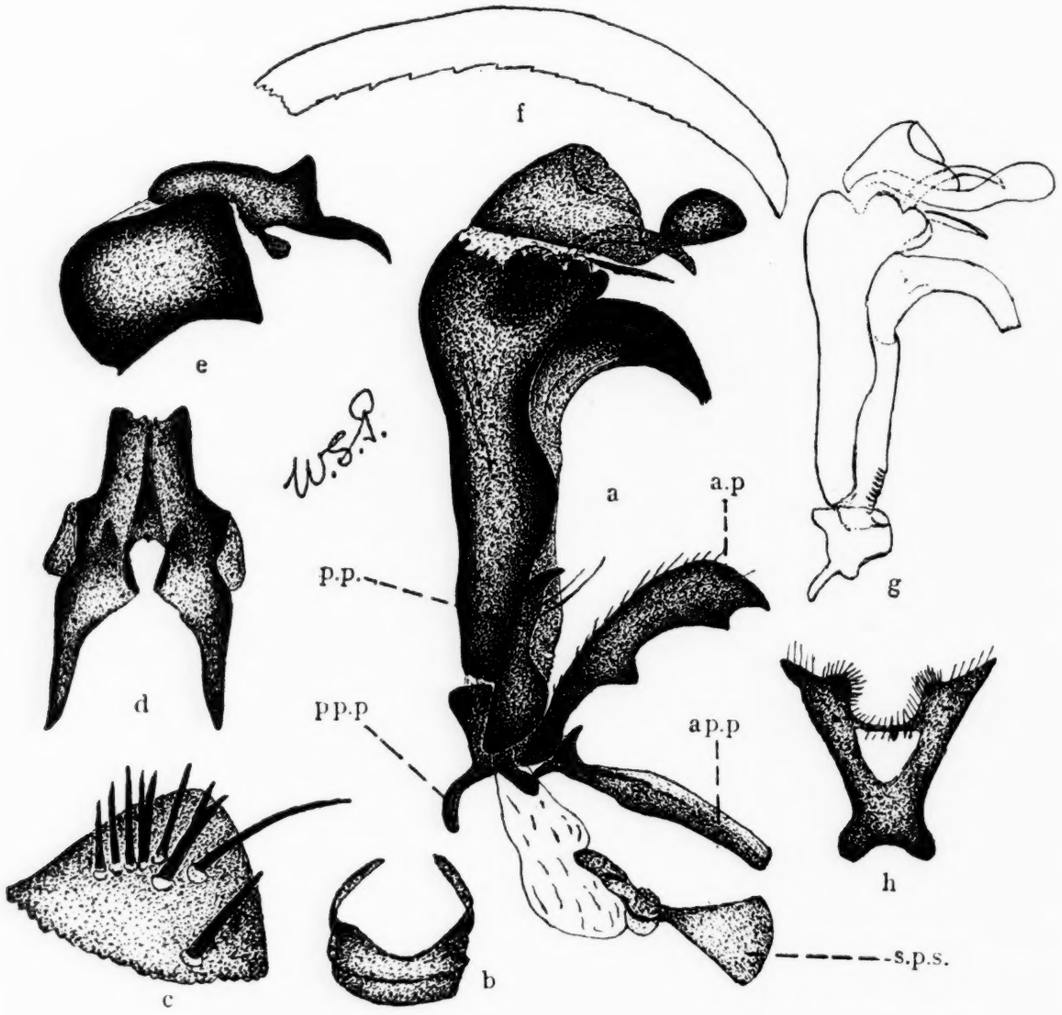


FIG. 6. *a*.—Phallosome and one paramere of *S. arno* in side view; lettering as in fig. 4, *a*; *b*.—Ninth tergo-sternum; *c*.—End of hump of anal cercus, showing bristles; *d*.—Ventral view of anal cerci and distal segments of ninth coxites; *e*.—Lateral view of tenth tergum, to show anal cercus and distal segment of ninth coxite in side view; *f*.—One of the serrated rods from end of phallosome; *g*.—Outline of phallosome, to show its structure; note the very short basal part; *h*.—Fifth sternum.

(fig. 4, *a.p.*) is a long plate ending in a short, sharp, beak-like point, and is expanded near its proximal end; it bears numerous short hairs on the inner surface; the posterior part of the paramere is rather narrow, short, upstanding, ending in a slightly bent point, and having two hairs near the end. The ninth

tergo-sternum is wide, the posterior arms being short and wide. Sternum 5 (fig. 4, *b*) is long and wide, the posterior arms are short and extended externally into pointed processes, and the rounded inner margins are armed with numerous short stiff hairs.

FEMALE. Fig. 5. Tergum 6 is divided into two distinct plates, their free dorsal ends sloping outwards and forming a characteristic notch (fig. 5, *e*). Tergum 7 is wanting. Sternum 5 is rectangular in shape. Sternum 6 is a wide convex plate, as shown in the illustration (fig. 5, *st. vi*). Sternum 7 is almost as wide and has rounded sides. Sternum 9 is very narrow and is attached at the distal end of sternum 7, its narrow sides being more strongly chitinized and appearing as rods. Sternum 10 (fig. 4, *a*, *c*) is a large triangular-shaped plate covered with numerous fine hairs; attached to its proximal side, and forming the dorsal wall of the genital opening, there is a large accessory plate (fig. 5, *c*) with somewhat produced sides. The signum is large and strongly chitinized, as shown in fig. 5, *c*.

Sarcophaga arno Curran. We are indebted to Mr. E. G. Gibbins for a long series of specimens, which were bred from human faeces at Katosi, Lake Victoria, Uganda, and which we take to be *S. arno* Curran. Unfortunately Curran's drawing of the male terminalia is small, and the structure of the phallosome and parameres is not detailed enough for us to be certain of our identification; we, therefore, identify this species as *arno* with some doubt.

SALIENT DIAGNOSTIC CHARACTERS OF TERMINALIA. MALE. Fig. 6. The anal cercus (fig. 6, *e*) when seen in side view is very similar in shape to that of *haemorrhoidalis*; the ventral hump is, however, very much wider and longer and is armed with many small stout bristles; the free end is narrower, slightly longer, and bends in a gentle curve to the pointed end; the posterior part, where it is joined to its fellow, as seen in ventral view (fig. 6, *d*), is much wider. The phallosome is structurally similar, the basal part small and wide, the posterior process distinctly longer; the distal part is wider, and the hood at the end shorter and wider. The proximal end of the hood is extended into a process which is roundly expanded at the end, the shape of the expanded part varying according to the position from which it is viewed (fig. 6, *a*, *g*); the other two processes are almost exactly similar to those of *haemorrhoidalis*: the more proximal one narrow and pointed in side view, but expanded when seen from the ventral surface; the other a serrated rod, as is seen in the enlarged drawing. The anterior paramere (fig. 6, *a*) when seen in side view appears as a wide plate with a deep emargination at the end, which is pointed; it has many short hairs along the inner surface; the posterior part of the paramere is a short plate, very similar to that of *haemorrhoidalis*, but distinctly wider at the base and with two hairs near the pointed end. The ninth tergo-sternum (fig. 6, *b*) is very similar to that of *haemorrhoidalis*. Sternum 5 (fig. 6, *h*), too, is structurally similar to that of *haemorrhoidalis*, but the lateral processes at the distal end are longer.

FEMALE. Fig. 7. A comparison of the illustration of the female terminalia of *haemorrhoidalis* (fig. 5) with that of the terminalia of the female *arno* (fig. 7) clearly shows that they are structurally similar. In both, sterna 6, 7 and 9 are very similar, especially sterna 7 and 9; the latter is attached at the end of sternum 7 in the same way as in *haemorrhoidalis* and is only slightly wider, the sides being not so strongly chitinized. In both species there is an accessory plate attached to sternum 10, which is triangular in shape, and in both the signum is a strongly chitinized structure, that of *arno* differing in shape from that

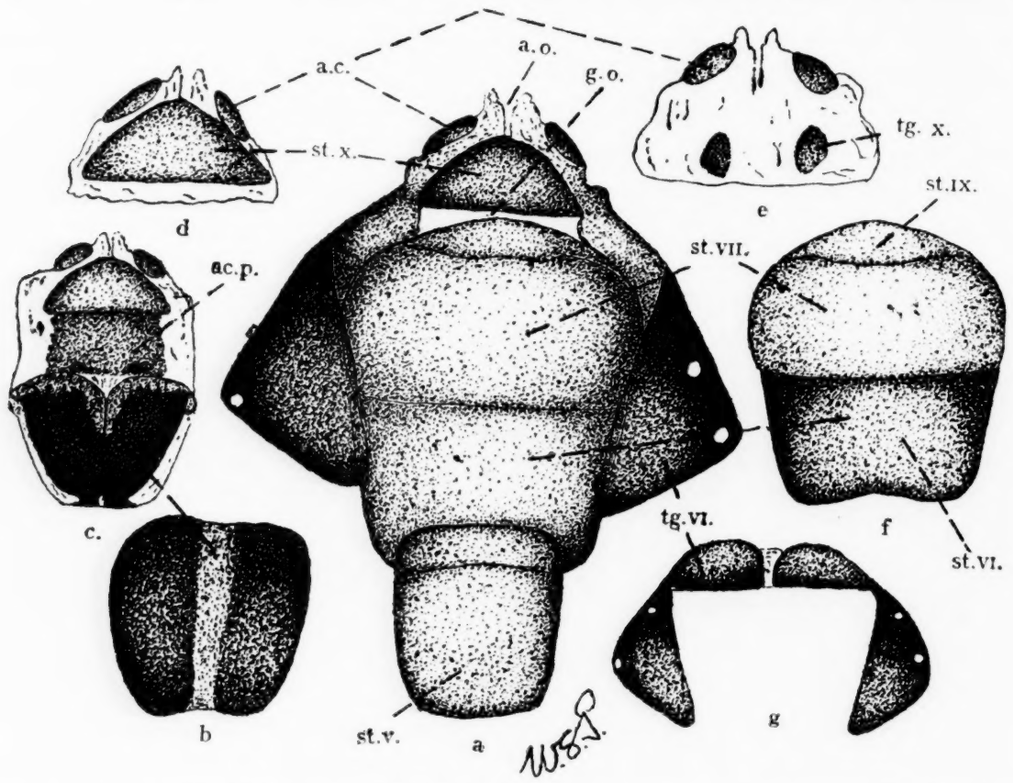


FIG. 7. *a.*—Ventral view of terminalia of female *S. arno*; *g.o.*—Genital opening; other lettering as in fig. 5, *a*; *b.*—Signum, showing difference in structure; *c.*—Ventral view of end of terminalia, to show anal cerci, tenth sternum, accessory plate and signum; *d.*—Ventral view of end of terminalia, showing anal cerci and tenth sternum; *e.*—Dorsal view of end of terminalia, to show tenth tergum in two parts; *f.*—Sixth, seventh and ninth sterna; *g.*—Sixth tergum, to show its two parts.

of *haemorrhoidalis*. In both species tergum 6 is divided into two plates, the notch between the two not being so well marked in *arno* as in *haemorrhoidalis*. In both there are two small plates in the position of tergum 9.

Sarcophaga arnoides sp. nov. Fig. 8. This species, one specimen of which was sent to us from Prospect by Mr. Munro, is another closely allied species. As noted above, it is not possible to be absolutely certain of the true identity of *S. arno* Curran, and it is just possible that this species is *arno*; but, in any case, in the meantime we shall regard it as a new species; we are, however,

certain that it is distinct from what we have taken to be *S. arno*. We give here some notes on this species and compare it with *S. haemorrhoidalis* and *S. arno*.

MALE. *Head.* Width of frons 0·80 mm., or 0·53 of an eye-width ; frontal stripe well defined, narrowing towards vertex ; two parafrontalia together about the width of frontal stripe. Lateral verticals wanting. Frontals 11, the uppermost one strong, directed upwards, the lowest five strongly diverging. Parafrontalia, parafacialia and cheeks silvery. Hairs on parafrontalia and parafacialia of fair size, 4 or 5 stronger hairs along the lower margin of each eye. Cheeks anteriorly black haired, posteriorly pale, soft haired. Back of head with 3 rows of black hairs. Antennae and palpi black.

Thorax. Acrostichals present as prescutellars. Posterior dorso-centrals 5, all well differentiated except for the anterior one. Intra-alars 1 : 2. Scutellum with the usual bristles.

Legs. Femora not fringed. Hind tibiae sparsely fringed on posterior side. All tibiae more or less brownish in colour.

Wing. Length 10 mm. Genital segments both red.

HOLOTYPE. Male. Pt. Shepstone, August 19th, 1920 (H. K. Munro).

TABLE

Showing the comparison of the external characters of the three species belonging to the *haemorrhoidalis* group

	haemorrhoidalis	*arno*	*arnoides*
Frons	Broad, 1·0 mm., or 0·65 of eye-width	Narrow, 0·59 mm., or 0·41 of eye-width	0·80 mm., or 0·53 of eye-width
Lateral verticals	Weakly developed	Not differentiated	Not differentiated
Colour of face	Golden	Dirty golden	Silvery
Cheek	Black haired anteriorly ; pale soft haired posteriorly	Same as in *haemorrhoidalis*	Same as in *haemorrhoidalis*
Prescutellars	Absent	Absent or very weak	Well differentiated
Posterior dorso-centrals	Only the posterior 2 or 3 strong ; anterior ones weak	Same as in *haemorrhoidalis*	Better developed; except the anterior one, all rather strong
Tibiae	Black; hind tibiae fringed on both sides	Brownish ; hind tibiae fringed on both sides	Brownish ; hind tibiae fringed on posterior side only
Genital segments	Red ; first segment with 2 pairs of marginals	Light orange; first segment without marginals	Red ; first segment without marginals

SALIENT DIAGNOSTIC CHARACTERS OF TERMINALIA. MALE. Fig. 8. The anal cercus (fig. 8, *e*) is very similar to that of *S. arno* (fig. 6, *e*) ; the free end, however, is distinctly shorter and wider when seen in side view, and the ventral hump has much longer bristles ; in ventral view the differences between the free

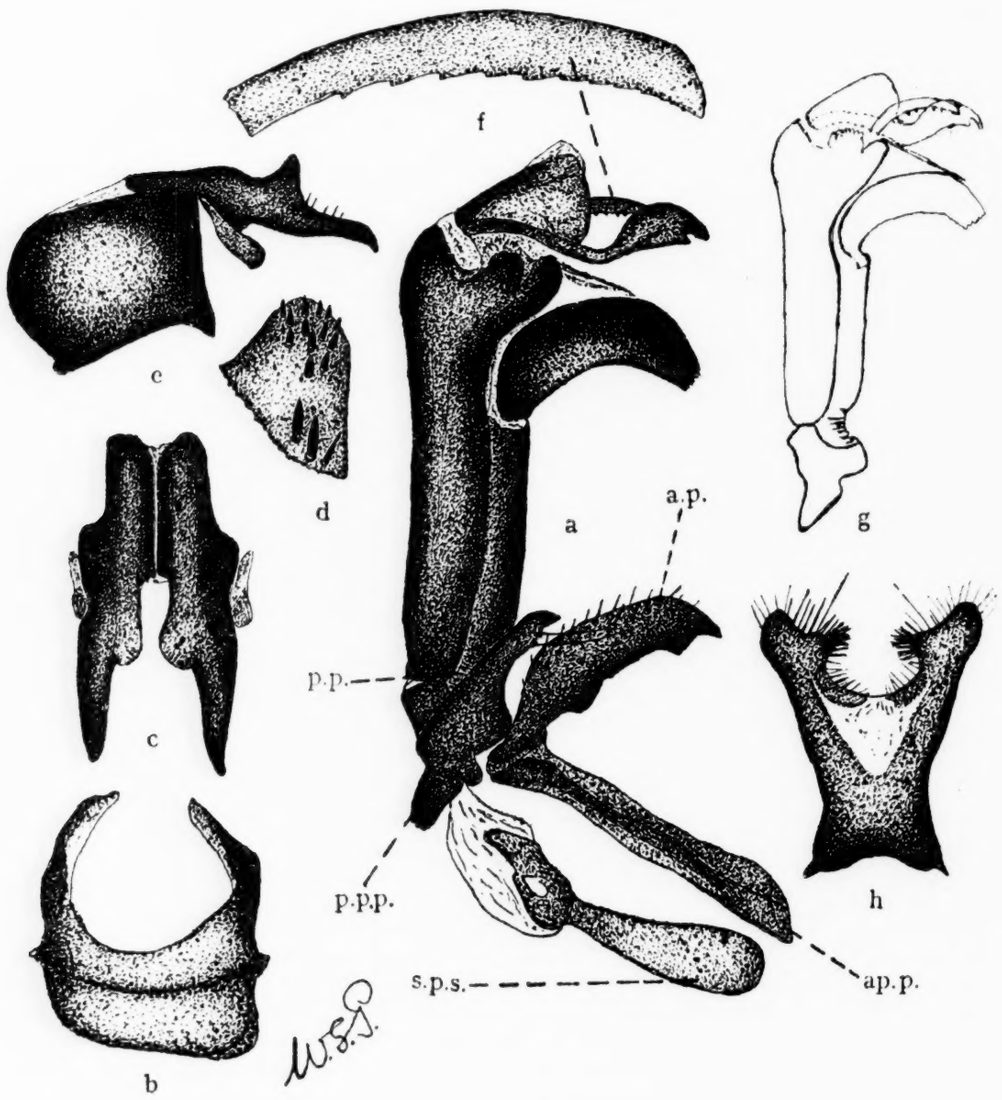


FIG. 8. *a.*—Phallosome and one paramere of *S. arnoides* in side view ; lettering as in fig. 4, *a* ; *b.*—Ninth tergo-sternum ; *c.*—Ventral view of anal cerci and distal segments of ninth coxites ; *d.*—End of hump of anal cercus, to show short bristles ; *e.*—Lateral view of tenth tergum, anal cercus and distal segment of ninth coxite ; *f.*—One of the serrated rods from the end of the phallosome ; *g.*—Outline of phallosome, to show its structure ; *h.*—Fifth sternum.

end of the anal cercus is well shown by comparing fig. 8, *c* with fig. 6, *c*. The phallosome is structurally similar, but there are some marked differences. The posterior process is shorter ; the hood at the end of the distal part is distinctly smaller, and the finger-like process has a notch on it at the end. There is

practically no difference between the parameres of the two species. The fifth sternum (fig. 8, *h*) of *S. arnoides*, though structurally similar, differs in that the outer ends of the posterior lobes are rounded, whereas in *S. arno* they are pointed. Taking these differences into account, as well as those of the external characters noted above, we have no doubt that this species is distinct from *S. arno*, and we therefore propose to name it *S. arnoides*. We have not had an opportunity of examining the female terminalia.

TERMINALIC CHARACTERS OF THE *HAEMORRHOIDALIS* GROUP

In this small group the characters of the terminalia are as follows. MALE. The phallosome has a small proximal part to which is attached the long, strongly chitinized, distal part ending in a characteristic semi-chitinous hood, a large serrated plate on the ventral side, a smaller one above it and a serrated rod-like plate ; the hood may have a lateral, long, finger-like process expanded at the end. The anal cerci are long and bent at the end, and may have a ventral hump armed with bristles. FEMALE. Tergum 6 is divided into two distinct plates ; sterna 6 and 7 are wide convex plates, while sternum 9 is a short wide plate attached to the distal end of sternum 7. There is an accessory plate attached to sternum 10, and the sigmum is well developed.

It will be noted that the phallosome of *Sarcophaga robusta* is somewhat similar, in that it has a small proximal part and a long distal portion ; but the similarity ends there. It may be that this is a primitive type of phallosome in the genus.

REFERENCES

ALDRICH, J. M. (1916). *Sarcophaga* and allies in North America. La Fayette, Indiana.

BÖTTCHER, G. (1912-13). Die männlichen Begattungswerkzeuge bei dem Genus *Sarcophaga* Meig. und ihre Bedeutung für die Abgrenzung der Arten. *Deutsch. Ent. Zeitschr.* ; 1912 : Hft. 5, 525, Hft. 6, 705 ; 1913 : Hft. 1, 1, Hft. 2, 115, Hft. 3, 239, Hft. 4, 351.

CURRAN, C. H. (1934). Sarcophaginae of the American Museum Congo Expedition (Diptera). *Amer. Mus. Novit.*, no. 727.

HARDY, G. H. (1927). Notes on Australian and exotic Sarcophagid flies. *Proc. Linn. Soc. N.S.W.*, **52**, 447.

——— (1934). Notes on Sarcophagid flies. *Austr. Zool.*, **8**, 50.

HO, C. (1932). Notes on Sarcophagid flies, with description of new species. I : *Albiceps*-group. *Bull. Fan Mem. Inst. Biol.*, **3**, 345.

JOHNSTON, T. H., and HARDY, G. H. (1923). A revision of the Australian Diptera belonging to the genus *Sarcophaga*. *Proc. Linn. Soc. N.S.W.*, **48**, 94.

PARKER, R. R. (1919). Concerning the subspecies of *Sarcophaga dux* Thomson. *Bull. Brooklyn Ent. Soc.*, **14**, 41.

——— (1923). New Sarcophagidae from Asia, with data relating to the *dux* group. *Ann. & Mag. Nat. Hist.*, *9th ser.*, **11**, 123.

PATTON, W. S., and WAINWRIGHT, C. J. (1935-37). The British species of the subfamily Sarcophaginae, with illustrations of the male and female terminalia. *Ann. Trop. Med. & Parasitol.*, **29**, 73, 517 ; **30**, 187, 337 ; **31**, 303.

SENIOR-WHITE, R. (1924). A revision of the sub-family Sarcophaginae in the Oriental region. *Rec. Ind. Mus.*, **26**, 193.

A NEW SPECIES OF NEMATODE (*SUBULURA LEACHII*) FROM A KINGFISHER

BY

A. KIRSHNER

(*From the Department of Parasitology, Liverpool School of Tropical Medicine*)

(*Received for publication May 6th*, 1938)

SUPERFAMILY **Subuluroidea** TRAVASSOS, 1930

FAMILY SUBULURIDAE YORKE AND MAPLESTONE, 1926

SUBFAMILY SUBULURINAE TRAVASSOS, 1914

Genus *Subulura* Molin, 1860.

SUBULURA LEACHII SP. NOV.

Several males and females were obtained in New Guinea from the intestine of *Dacelo leachi*. They are the first species of *Subulura* recorded from this family of birds.

The worms are slender, and the cuticle is marked with fine transverse striations. They vary in length from 1 cm. to 1·4 cm., and the anterior extremity is slightly bent laterally. Cervical alae are present and extend along each side from the mouth to a point beyond the middle of the body, narrowing as they pass posteriorly. The lips are inconspicuous and the head is provided with 6 papillae, 2 being lateral and 4 subventral. The buccal capsule is well chitinized, almost as broad as it is long, and has three teeth at its base (figs. 1, 2). The oesophagus is curved slightly in conformity with the bent anterior extremity, and ends in a club-like dilatation. It is separated from the round oesophageal bulb by a definite transverse constriction (fig. 3).

MALES. These measure about 1 cm. in length, and have a maximum diameter of about 400μ. The buccal capsule is 40μ in length, 30μ in width, and the average thickness of the chitinous walls is about 6μ. The length the oesophagus (without bulb) is 1·2 mm. The bulb is almost globular, with a diameter of 250μ. The nerve ring is situated 330μ from the anterior extremity.

The genital sucker is oval in shape, surrounded by well-marked muscle fibres, and measures about 230μ in length ; its posterior border is situated 700μ from the tip of the tail. There is no chitinous rim attached to the sucker.

The tail is curved ventrally and measures 290μ in length (fig.4), terminating in a fine point. The spicules are equal, alate at their proximal extremity, and measure from 1·25 mm. to 1·35 mm. in length. The gubernaculum is well developed, somewhat triangular in shape, and 200μ in length.

There are 11 pairs of sessile papillae present, of which 3 pairs are preanal, 2 pairs adanal, and 6 pairs postanal (fig. 5). Narrow caudal alae are present.

FEMALES. These measure about 1·4 cm. in length, the greatest diameter being 500μ. The buccal capsule is 46μ in depth and 38μ in width. The total length of the oesophagus is 1·55 mm., the oesophageal bulb comprising 250μ of its length.

The nerve ring is situated 370μ from the anterior extremity. The tail is pointed, and the distance from the anus to the tip of the tail is 960μ.

The vulva is placed just anterior to the middle of the body. The eggs measure 54μ in length by 34μ in breadth, and show various stages of development ; the fully mature egg contains a larva.

The genus *Subulura* includes some 50 species, most of which are parasitic in birds. The following species have been recorded from primates : *S. jacchi*, *S. distans*, *S. sarasinorum*, *S. malayensis*, *S. neodistans*, *S. prosimiae*, *S. otolicni* (= *S. loveridgei*), *S. pararmata* and *S. cynomolgi*. A few species are found in rodents, whilst *S. paramelis* and *S. interrogans* have been described from marsupials.

The following table serves to differentiate *Subulura leachii* sp. nov. from all other species of this genus in which the males possess 11 pairs of papillae and spicules of equal length :—

	Length of spicules	Length from posterior end of sucker to tip of tail	Length of male tail
S. leachii sp. nov.	1·25–1·35 mm.	700μ	290μ
S. halli	1·5 mm.	1·2 mm.	500μ
S. galloperdicis	760–800μ	860μ	210μ
S. travassosi	1·71 mm.	508μ	127μ
S. olympioi	899μ	465μ	228μ
S. trogoni	558μ	423μ	232μ
S. strongylina	1·18 mm.	450μ	186μ
S. suctoria	1·02–1·15 mm.	500μ	211μ
S. similis	Probably identical to *S. suctoria*	—	—
S. taiwanensis	770–860μ	?	220–260μ
S. hindi	910μ	312μ	244μ
S. otolicni (= *S. loveridgei*)	1·8 mm. (v. Beneden) 1·55 mm. (Baylis)	—	About 360μ
S. jacchi	1·694 mm.	—	—

All the above species are from birds, with the exception of the last three.

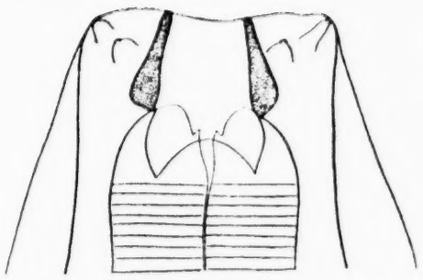


FIG. 1. *Subulura leachii* sp. nov. Head, dorsal view. (× 330.)

FIG. 2. *Subulura leachii* sp. nov. Head, lateral view. (× 330.)

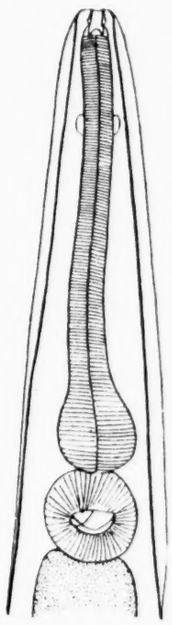


FIG. 3. *Subulura leachii* sp. nov. Anterior extremity, dorsal view. (× 45.)

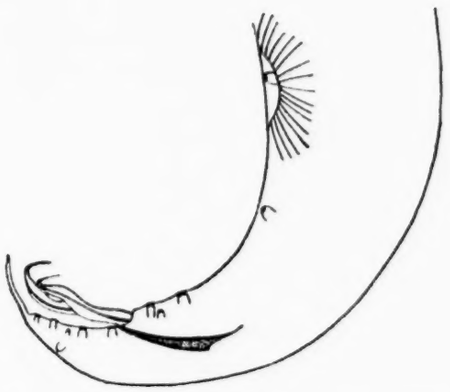


FIG. 4. *Subulura leachii* sp. nov. Tail of male, lateral view. (× 75.)

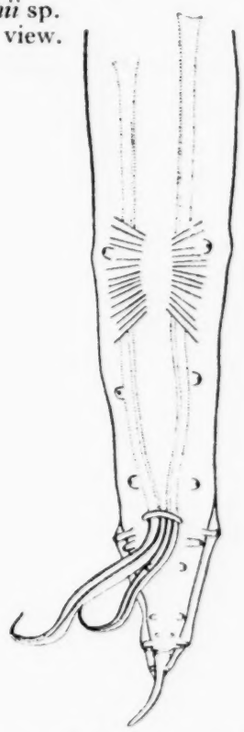


FIG. 5. *Subulura leachii* sp. nov. Tail of male, ventral view. (× 75.)

REFERENCES

BAYLIS, H. A. (1930). Some Heterakidae and Oxyuridae [Nematoda] from Queensland. *Ann. & Mag. Nat. Hist.*, 10*th ser.*, **5**, 354.

——— (1936). The fauna of British India, including Ceylon and Burma. Nematoda. vol. 1 (Ascaroidea and Strongyloidea). London.

CAMERON, T. W. M. (1930). The species of *Subulura* Molin in primates. *Jl. Helminthol.*, **8**, 49.

CRAM, E. B. (1927). Bird parasites of the nematode suborders Strongylata, Ascaridata, and Spirurata. *Bull. U.S. Nat. Mus.*, no. 140.

LEE, C. U. (1930). *Subulura malayensis* n. sp., a new nematode from two Malayian monkeys. *Zentralbl. f. Bakt., Orig.*, **116**, 169.

MAPLESTONE, P. A. (1931). Parasitic nematodes obtained from animals dying in the Calcutta Zoological Gardens. pt. 4-8. *Rec. Ind. Mus.*, **33**, 71.

MIRZA, M. B. (1936). *Subulura hindi* n. sp., a new nematode parasite of *Sciurus palmarum*. *Proc. Ind. Acad. Sci., B.*, **3**, 125.

SPAUL, E. A. (1929). Two new nematode bird parasites from Central Asia. *Ann. & Mag. Nat. Hist.*, 10*th ser.*, **4**, 451.

YAMAGUTI, S. (1935). Studies on the helminth fauna of Japan. pt. 12 : Avian nematodes, I. *Jap. Jl. Zool.*, **6**, 403.

YORKE, W., and MAPLESTONE, P. A (1926). The nematode parasites of vertebrates. London.

STUDIES IN CHEMOTHERAPY*

XVIII.—CHANGES IN THE BLOOD AND URINE PRODUCED BY ADMINISTRATION OF UNDECANE DIAMIDINE

BY

J. DEVINE

(*Received for publication May 17th*, 1938)

From a recent review by Lourie and Yorke (1937) of the evidence dealing with the curative action of synthalin (n-decane 1 : 10 diguanidine) on trypanosome-infected mice, it appears that the effect was ascribed by earlier workers (Jancsó and Jancsó, 1935; Schern and Artagaveytia-Allende, 1935, 1936) chiefly to the hypoglycaemic activity of synthalin on the host, and only in less degree to its direct toxicity for parasites. Lourie and Yorke (1937) showed, however, that, whereas synthalin exerts a powerful trypanocidal effect *in vitro*, the corresponding activity of insulin is negligible, and they concluded that the therapeutic effect of synthalin resides in a direct lethal action. It was later shown by King, Lourie and Yorke (1937), in an examination of a large number of compounds of related chemical structure, that a pronounced *in vitro* action is exhibited by certain of the aliphatic diamidines, of which the most active, n-undecane 1 : 11 diamidine, is less toxic to the host than synthalin, but equally trypanocidal.

The effects of administration of guanidine derivatives, and especially of synthalin, to experimental animals have been examined in detail by a large number of workers. Bischoff, Sahyun and Long (1929) showed that hypoglycaemic activity was associated principally with long chain aliphatic basic derivatives, but that a marked effect was seldom obtained by less than the lethal dose. Hypoglycaemic activity, lethal dose and liver damage ran nearly parallel, whereas kidney damage was effected quite independently of hypoglycaemic activity by both aromatic and aliphatic compounds. Synthalin (the most active of the diguanidines) was reported by Blatherwick, Sahyun and Hill (1927) to bring about extensive renal and hepatic injury, and it was suggested that the onset of hypoglycaemia depends partly on the breakdown of the normal mechanism of glyconeogenesis. This fundamental difference between the action of synthalin and of insulin was later confirmed by Bodo and Marks (1928), who showed that in synthalin hypoglycaemia the hyperglycaemic response to adrenaline is abolished, and that the glucose disappearing from the liver is, in the main, not directly combusted or laid down as muscle glycogen, but is probably first converted to lactic acid.

*This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council.

The alkylene diamidines have been less well examined. According to Broom (1936) they closely resemble the diguanidines, but do not affect carbohydrate metabolism so readily; the hypoglycaemic activity in this series rises to a maximum in the case of the octane derivative (sebacamidine), whereas undecane diamidine produces only a prolonged and fatal hyperglycaemia.

In view of the promising results mentioned above in the use of undecane diamidine against trypanosome infections, a more precise study has been made of this compound, so that ultimately an upper limit may be fixed to the amount which can be administered without serious renal or hepatic injury.

EFFECT OF UNDECANE DIAMIDINE ON RABBITS

A. Administration of Sub-Lethal Doses

Experiment I. Two rabbits (A and B) were kept in metabolism cages and fed on a routine diet of oats and cabbage each day between 5 and 7 p.m. Full-day urines were collected under toluene at 11 a.m. In order to obtain some idea of the normal variations, analyses* were made of fasting blood samples collected on two successive days and on the full-day urines of the same days (see first portions of Tables I and II).

With a view to ascertaining the effect of a sub-lethal dose of the drug, 5 mgm. per kilo. (about half the minimum lethal dose) was injected intravenously, and frequent analyses of the fasting blood were made during the following 30 hours. At the end of this period, normal routine feeding was resumed, but further fasting blood samples were examined 49 and 54 hours respectively after the injection, to determine whether there were any delayed effects.

The results, which are summarized in the second portion of Table I, show that the administration of the drug in a dose of 5 mgm. per kilo. was not followed by any significant change in the blood sugar values. Only in a single instance (Rabbit A, 49 hours after injection) is there any suggestion of a slight tendency towards hyperglycaemia—according to Cammidge (1927) the maximum glycaemic response of rabbits to synthalin is not attained until after 20 hours. This slight rise in sugar content exhibited by Rabbit A is, however, probably of no significance, because this rabbit appeared to show greater tolerance to the drug than Rabbit B, as evidenced by the figures for nitrogen retention. Both rabbits showed a definite increase in blood urea which appeared to reach its

*The methods of blood sugar and urea analyses used were those of Fujita and Iwatake (1931) and of Conway (1933) respectively; non-protein nitrogen (N.P.N.) was determined by micro-Kjeldahl analysis of the Folin-Wu filtrate. In qualitative tests on urines, traces of 'sugar' (Benedict's test) and protein (sulphosalicylic acid and 'boiling' tests) were sometimes shown by controls, due partly, no doubt, to contamination of the urine with food particles, etc. Similar traces in experimental urines are, therefore, entered as a negative response.

TABLE I

Blood analyses after injection of 5 mgm. per kilo. undecane diamidine

Rabbits. *A*, ♀, 2·7 *kgm.* *B*, ♂, 1·5 *kgm.*

	Date	Time	Sugar mgm. per cent.		Urea mgm. per cent.		N.P.N. mgm. per cent.	
			A	B	A	B	A	B
Controls	17.12.37	12.15 p.m.	140	132	31·4	33·0	38·7	
		3.15 p.m.	130	119	32·7	29·9	40·1	
	18.12.37	11.15 a.m.	125	115	25·4	—	37·1	
		3.0 p.m.	108	120	32·7	—	—	
		5.15 p.m.	118	113	32·4	—	39·0	
	5.1.38	Injection 10.0 a.m.	128	129	—	—	42·0	—
		11.15 a.m.	136	115	—	—	41·2	43·9
		1.0 p.m.	117	118	33·0	49·3	44·4	48·7
		4.0 p.m.	129	109	36·3	61·9	42·2	57·2
		6.30 p.m.	119	119	36·1	71·3	45·0	62·1
		9.0 p.m.	132	126	—	—	47·0	—
	6.1.38	11 a.m.	119	115	40·3	69·5	48·1	62·0
		4·0 p.m.	115	114	—	—	43·4	60·0
	7.1.38	11.0 a.m.	151	125	26·8	29·8	40·6	42·6
		4.0 p.m.	140	128	—	—	37·1	—

maximum within the latter half of the first day ; the figures returned to normal within the second day after injection. The relatively smaller change in N.P.N. shows a similar trend.

TABLE II

Urine analyses in Experiment I

Date of collection		Vol. (c.cm.)	Urea		Total N		Urea N / Total N
			Gm. per cent.	Output (gm.)	Gm. per cent.	Output (gm.)	
Controls A	17–18.12.37	140	1·55	2·17	0·89	1·24	0·82
	18–19.12.37	87	1·81	1·58	1·06	0·92	0·80
Controls B	17–18.12.37	103	0·74	0·76	0·41	0·42	0·85
	18–19.12.37	125	—	—	0·64	0·80	—
A	6–7.1.38	160	2·27	3·63	1·28	2·05	0·83
	7–8.1.38	243	1·41	3·43	0·70	1·71	0·94
B	6–7.1.38	105	2·39	2·51	1·33	1·40	0·84
	7–8.1.38	89	1·55	1·38	0·96	0·86	0·75

As it has been necessary, in order to follow the changes in specimens of fasting blood, to starve the animals for about 15 hours before the injection and for 30 hours afterwards, analyses of the urine during this period would have had no value. Consequently, the urine analyses shown in the second part of Table II refer to the full-day samples collected on the two days following the period of starvation. If the effects of temporary suppression of urine secretion during the period of starvation be taken into account, the figures obtained suffice to indicate the absence of any gross or lasting kidney dysfunction. The urines were in all cases free from any but insignificant traces of protein or reducing substances.

Administration of sufficiently high, but well-tolerated, doses of undecane diamidine is followed, both in rabbits and in man, by the symptoms of nausea and anorexia which are common to many of the so-called renal poisons. The resultant variation in food intake makes accurate day-to-day interpretation of the nitrogen balance, and therefore of renal efficiency, virtually impossible.

Experiment II. In an effort to minimize these effects in a fresh pair of animals, the diet was slowly reduced to little more than half the stock amount, and then maintained from a period five days prior to the experiment, so that N-equilibrium could be reached. After administration of the same dose of drug as in Experiment I, however, only a small fraction of the reduced diet was taken ; a second injection was therefore made on the following morning to induce greater changes in the urine. The constant diet was presented each day, food residues from the previous day being left in the cages until appetite was normal. Full-day urines were collected at a later hour each day, according to the amount of diet eaten previously. Details of the blood and urine analyses are given in Table III.

The colour tests of Fouchet (blood serum) and of Ehrlich and Schlesinger (urine) for hepatic dysfunction were also carried out.

The blood picture is predominantly the same as that found in Experiment I. While the blood sugar again shows no significant change, a rise in urea is recorded comparable with that previously obtained.

Considering the reduced intake of nitrogen, and especially of water following on the first injection, the minimum figures recorded for the corresponding urines cannot be taken to indicate serious renal injury. Under these circumstances, and in the absence of albuminuria, the percentage of urea in the samples (1·64 and 2·94 per cent.) are sufficient guarantee of the concentrating power of the kidneys. Moreover, the urines corresponding to the second injection show, with little diminution in nitrogen concentration, a big rise in volume, exceeding the secretion for the following days, so that the nitrogen output is almost equivalent in both cases to the mean output of the next four days. This corresponds to the mildly nephritic state in which the nitrogen output is almost normal, but requires for its secretion a bigger ' head ' in the blood.

TABLE III

Blood and urine analyses after injection of 5 mgm. per kilo. undecane diamidine

Rabbits. *C*, ♂, 2·3 *kgm.* *D*, ♂, 2·3 *kgm.*

A. BLOOD ANALYSES

Time of injection	Time of sample	Sugar mgm. per cent.		Urea mgm. per cent.		N.P.N. mgm. per cent.	
		C	D	C	D	C	D
15.3.38. 10.0 a.m.	Before injection	90·5	89·3	23·9	25·4	39·3	46·2
	12.15 p.m.	85·6	94·3	32·8	36·7	37·6	49·8
	3.30 p.m.	89·8	92·4	48·3	56·5	50·1	47·0
	4.30 p.m.	89·4	94·1	54·2	56·6	51·3	58·0
16.3.38. 10.0 a.m.							

B. URINE ANALYSES

Time of injection	Time of collection	Vol. (c.cm.)	Urea		Total N		Urea N / Total N
			Gm. per cent.	Output (gm.)	Gm. per cent.	Output (gm.)	
	C 14th 10.0 a.m.	98	2·29	2·25	1·39	1·37	0·77
	C 15th "	61	—	—	1·49	0·91	—
	D as C "	82	2·30	1·88	1·37	1·12	0·78
	D as C "	52	4·04	2·11	2·24	1·17	0·84
15.3.38 10.0 a.m.	C 16th 10.0 a.m.	35	1·64	0·57	1·10	0·39	0·70
16.3.38 10.0 a.m.	C 17th 2.30 p.m.	88	—	—	1·01	0·89	—
	C 18th 4.30 p.m.	51	2·86	1·46	1·45	0·74	0·92
	C 19th 3.30 p.m.	54	3·75	2·03	2·21	1·19	0·79
	C 20th 1.30 p.m.	72	(1·06)	(0·76)	1·48	1·07	(0·33)
	C 21st 10.0 a.m.	77	1·57	1·21	0·94	0·72	0·78
	D as C	32	2·94	0·94	1·83	0·59	0·75
	D as C	57	—	—	1·55	0·88	—
	D as C	44	5·18	2·28	2·50	1·10	0·97
	D as C	37	3·50	1·30	2·30	0·85	0·71
	D as C	47	2·89	1·36	1·83	0·86	0·74
	D as C	49	2·37	1·16	1·33	0·65	0·83

None of the qualitative tests of hepatic or renal injury was positive. In an isolated case the urine of one animal (Rabbit C, March 19th–20th, 1938) contained a large proportion of ammonium carbonate. Although collected as usual under preservative, it cannot be regarded as significant at this point.

B. Administration of Successive Small Doses

Experiment III. Examination was made of two groups of rabbits which had been infected with trypanosomes (*T. rhodesiense*) and then subjected to undecane diamidine treatment as follows:

Group I. Infected on September 29th, 1937. Ten doses of 1·25 mgm. per kilo. undecane diamidine daily from October 28th, 1937, followed by relapse; then a further ten doses from November 23rd, 1937.

Group II. Infected on November 5th, 1937. Ten doses of 2·5 mgm. per kilo. daily.

In the first group, fasting blood samples were analysed throughout the day after that on which the last injection was made, and in the second group on the same day as the last injection.

On the basis of control figures obtained from some of the same animals several months later, all the samples were practically normal. These doses thus appear to be well tolerated without leading to marked cumulative renal injury; but it must be borne in mind that, because of the very great reserve powers of the liver and kidneys, significant changes in chemical analyses of this type are only shown after relatively gross injury.

C. Administration of the Maximum Tolerated Dose

According to Broom (1936) the diamidines only affect the blood sugar level in lethal doses sufficient to bring about extensive renal and hepatic injury. They belong to one of two classes, and either produce a transient hyperglycaemia, followed by a fatal hypoglycaemia, or a prolonged hyperglycaemia, which, with very large doses, proves fatal. Thus, while sebacamidine (the most active member) produces hypoglycaemia in the range 1–2 M.L.D., the decane derivative in the same range produces hyperglycaemia, and only becomes hypoglycaemic above this dose; the minimum lethal dose of undecane diamidine (15 mgm. per kilo. subcutaneously) is stated to produce in rabbits only a hyperglycaemia, but these figures suggest that in appropriately graduated amounts it might be shown to cause hypoglycaemia before death.

The minimum lethal dose (intravenously) determined in this laboratory for infected rabbits is about 10 mgm. per kilo., but is probably higher for normal animals, in which this may be regarded as the maximum tolerated dose. The degree of hepatic stimulation or damage effected by this amount in fasting animals is measured in Table IV by parallel determinations of blood sugar and amino-N (method of Folin; see Peters and Van Slyke, 1931). The changes are contrasted with those for a dose of 5 mgm. per kilo. The assessment of the diminished ability of the liver to deaminize amino acids from determinations of blood amino-N is not invalidated, according to Blatherwick *et al.* (1927), by simultaneous injury to the kidneys.

TABLE IV

Comparison of blood analyses after injections of 5 mgm. per kilo. and 10 mgm. per kilo. undecane diamidine respectively

Rabbits. *C*, ♂, 2·2 *kgm.* (5 *mgm. per kilo.*). *D*, ♂, 2·2 *kgm.* (10 *mgm. per kilo.*)

Date	Time after injection	Sugar mgm. per cent.		Amino-N mgm. per cent.	
		C	D	C	D
4.4.38	Injection 10.0 a.m.	92·2	96·6	10·2	9·5
	12.30 p.m.	—	119·0	9·3	9·0
	2.30 p.m.	—	120·0	8·8	10·0
	5.0 p.m.	—	114·0	8·8	9·3
	10.0 p.m.	—	101·0	10·4	10·7
5.4.38	10.0 a.m.	85·8	90·8	9·8	8·7
	2.0 p.m.	90·0	101·0	9·8	8·8

The Fouchet test was negative in both cases.

With the smaller dose there is no sign of delayed hyperglycaemia or of liver derangement as measured by the amino-N level; the larger dose shows a slight hyperglycaemia, maximum at about 5 hours after injection, returning to normal the same day, whereas the amino-N still remained constant up to the 28th hour, when the blood urea measured 164 mgm. per cent.

Histological Examination

As Rabbit D showed no signs of approaching death after 28 hours, it was killed, and sections of kidney, liver, heart muscle and pancreas were submitted to examination by Dr. J. L. Dales, to whom the writer is indebted. There were no macroscopic changes. The liver, pancreas and heart muscle sections were perfectly normal. In the kidneys the collecting tubules and glomeruli were undamaged, but the convoluted tubules showed degenerative changes, varying from simple cloudy swelling to complete necrosis with disappearance of the cell outline and the nucleus.

D. ADMINISTRATION OF LETHAL DOSES

In Table V are recorded estimations of fasting blood sugar, amino-N and urea following single injections of 15 mgm. and 20 mgm. per kilo. undecane diamidine. Both animals died in coma after convulsions. Rabbit E, which received the smaller dose, died first after 4 hours, F survived 6 hours. In both cases the final blood samples were taken from the heart a few minutes before death.

TABLE V

Blood analyses after injection of lethal doses of undecane diamidine

Rabbits. *E*, ♀, 1·6 *kgm.* (15 *mgm. per kilo.*). *F*, ♂, 1·6 *kgm.* (20 *mgm. per kilo.*)

Time after injection	Sugar mgm. per cent.		Amino-N mgm. per cent.		Urea mgm. per cent.	
	E	F	E	F	E	F
Injection 10.0 a.m.	93·4	97·1	8·9	9·2	27·0	26·7
12.0 noon	55·3	176·0	9·7	10·4	34·4	34·8
2.0 p.m.	22·1	55·2	15·9	11·6	42·0	42·7
4.0 p.m.	—	29·6	—	14·6	—	40·6

The results closely resemble those due to synthalin. The blood urea does not rise progressively with an increasing dose; but with highly toxic doses, causing death within a few hours, the rise is relatively slight. Blatherwick *et al.* (1927) showed that, of two different rabbits given the same lethal injection of synthalin, one died within 7 hours, showing a maximum rise of only 9 mgm. per cent. urea, whereas the other animal, which survived nearly three days, had a final blood content of 192 mgm. per cent. The urea figures recorded in Table V are much less than those corresponding to the administration of an easily tolerated dose of 5 mgm. per kilo. (see Table III). This may well to a slight extent be accounted for by the retardation of urea production following on hepatic injury.

Damage to the liver is evident in both cases from the rise in amino-N and from the glycaemic effect. Thus, the more quickly fatal dose (15 mgm. per kilo.) caused an immediate hypoglycaemia accompanied by a rapid rise of amino-N. The larger but longer tolerated dose caused first a pronounced hyperglycaemia, followed then by a fatal hypoglycaemia, the rise in amino-N being continuous but less rapid than before.

Histological Examination

Liver and kidney sections were examined. Both animals showed cloudy swelling in the cells of the convoluted tubules of the kidneys. This was more marked in Rabbit E, which also exhibited degeneration of the liver cells ; these showed a granular cytoplasm with fat droplets around the central veins of the lobules. The liver sections of Rabbit F were normal.

EFFECT OF UNDECANE DIAMIDINE ON MAN

A report has recently been made by Glyn-Hughes, Lourie and Yorke (1938) of the therapeutic effects of undecane diamidine on induced malaria in cases of neuro-syphilis. As the effect of this drug on normal man could not

be studied, the opportunity was taken of examining the blood and urine of several of these cases during treatment.

In malaria, as in other febrile diseases, the increase in total metabolism, and especially in nitrogen metabolism, is known to lead to a negative nitrogen balance and sometimes to a rise of blood urea without any associated renal injury. Such factors must be taken into account in the present study of blood, and especially of urine, analyses. Barr and Du Bois (1918) have shown that, in malaria, even with adequate dietary protein, N-equilibrium is not immediately attained after the cessation of febrile paroxysms unless the calorific intake be high (3,000 cals.). If the latter be as low as 2,000 cals., there is a further delay of several days before equilibrium is accomplished. In the cases under consideration, with the small dietary intake which was acceptable, a marked negative balance was naturally found throughout the course of treatment. In these circumstances, analysis of urines, especially when the blood changes are very slight, may have little value, but it was thought that their inclusion is warranted by the fact that the opportunity of studying the effect of the drug on human patients would probably not be presented again.

In the first trial the patient (Mr. B.) was maintained as far as possible on the routine hospital diet, which was limited to the period 9.30 a.m.-4.0 p.m. so that fasting blood samples could be conveniently collected immediately before breakfast. Full-day urines were collected from 12 noon. One hundred mgm. undecane diamidine was given daily by mouth for 2½ days (50 mgm. at 10.0 a.m. and 10.0 p.m. each day). The results are outlined in Table VI.

TABLE VI

Blood and urine analyses after oral administration of 250 mgm. undecane diamidine to man during malaria

The drug was given in 50 mgm. doses at 10.0 a.m. and 10.0 p.m. on March 2nd and 3rd, and at 10.0 a.m. on March 4th, 1938

Date —.3.38	Blood			Urine					
					Urea		Total N		Urea N / Total N
	Sugar mgm. per cent.	Urea mgm. per cent.	N.P.N. mgm. per cent.	Vol. (c.cm.)	Gm. per cent.	Output (gm.)	Gm. per cent.	Output (gm.)	
(Control)2	78·3	21·8	21·3	1272	0·93	11·8	0·49	6·2	0·88
3	88·9	27·0	27·1	2065	0·76	15·6	0·46	9·5	0·77
4	80·0	24·5	24·3	1652	1·20	20·0	0·70	11·5	0·81
5	82·6	19·4	22·4	3230*	0·52	16·8	0·32	10·4	0·76

*Urine collected up to 10.30 a.m. instead of at noon.

The dose was obviously well tolerated, since there was a rise of only about 5 mgm. per cent. in blood urea, and the sugar level was not significantly disturbed. Thus, even if the extraneous factors due to the malarial condition may be supposed to have contributed to this slight urea retention, the total effect is practically negligible. In Table VII are recorded the results of treating two further cases at the same stage of the disease with an increased dose of undecane diamidine. The resultant increase in blood urea may confidently be ascribed almost wholly to the action of the drug.

In this second trial both patients (Mr. H. and Mr. W.) were given by mouth 75 mgm. undecane diamidine twice daily for 3 days—450 mgm. in all—which included an evening dose of 75 mgm. (on March 16th, 1938) before the first blood samples were taken on the following morning, so that at first no control analyses were immediately available. The effect on the appetite was more marked than before; an estimate of the resultant variations in protein and water intake was therefore made from notes kept of the case, and is given against the urine analyses. In such circumstances, when the net absorption of drug may vary considerably, more erratic results are to be expected than when it is administered intravenously.

TABLE VII

Blood and urine analyses after oral administration of 450 mgm. undecane diamidine to man during malaria

The drug was given in 75 mgm. doses at 10.0 p.m. on March 16th, 10.0 a.m. and 10.0 p.m. on March 17th and 18th, and 10.0 a.m. on March 19th, 1938

Date, —.3.38		Blood			Urine						Protein intake as urea (gm.)	H_2O intake (c.cm.)
		Sugar mgm. per cent.	Urea mgm. per cent.	N.P.N. mgm. per cent.	Vol. (c.cm.)	Urea Gm. per cent.	Urea Output (gm.)	Total N Gm. per cent.	Total N Output (gm.)	Urea N / Total N		
16	H.										8·7	700
	W.										8·7	900
17	H.	71·6	33·0	29·4	790	2·11	16·7	1·21	9·6	0·81	5·5	1080
	W.	74·4	35·3	29·4	600	3·04	18·2	1·66	10·0	0·85	11·1	960
18	H.	61·1	29·5	28·8	900	2·19	19·7	1·20	10·8	0·85	6·2	840
	W.	78·8	35·8	37·8	280	3·47	9·7	1·89	5·3	0·86	1·3*	300
19	H.	69·1	34·8	35·4	897	2·04	18·3	1·19	10·7	0·80	2·6	500
	W.	71·2	—	32·7	495	2·96	14·7	1·69	8·4	0·82	9·7	410
20	H.	82·5	43·1	37·9	550	2·30	12·7	1·37	7·5	0·78	0	500
	W.	82·1	37·6	31·4	525	3·13	16·4	1·95	10·2	0·75	9·3	1150
21	H.	79·8	29·2	28·9	942	1·54	14·5	0·82	7·8	0·87	6·3	850
	W.	79·1	25·0	29·9	257	3·07	7·9	1·66	4·3	0·86	8·3	890
22	H.				880	1·16	10·2	0·74	6·5	0·74		
	W.				440	1·53	6·73	0·93	4·1	0·77		

*Breakfast vomited; lunch refused.

The maximum figures for urea retention are shown on the first day after that on which the last dose was given (March 20th); on the second day following (March 21st), the blood urea had fallen rapidly to 29·2 mgm. per cent. and 25·0 mgm. per cent. respectively, which can be regarded as approximating to the normal figures before treatment. The blood sugar level was still unaffected.

Consideration of the urine analyses does not indicate any marked renal dysfunction. In Mr. W. the volume of urine was very erratic as compared with the water intake, but the variations do not seem to be related in any consistent manner to the drug administration or its effect on the blood. The percentage output of urea and total-N remained in both cases fairly constant until the last day (March 21st), when, as was to be expected, there was an approach to nitrogen equilibrium with a subsequent fall in nitrogen output. Tests for albuminuria and glycosuria remained negative.

DISCUSSION

The results obtained with rabbits broadly resemble those due to the synthalin homologues. The blood urea is more than doubled by an easily tolerated dose of 5 mgm. per kilo. undecane diamidine, but disturbance of the carbohydrate metabolism only becomes evident when the maximum tolerated dose is reached (10 mgm. per kilo.). Above this amount, lethal doses of undecane diamidine which produce death in a few hours (presumably by central action) cause relatively slight kidney damage but begin to affect the liver with a subsequent rise in blood sugar and amino-N. If we arrange the results of various doses in order (by survival time in the case of lethal doses), the glycaemic effects, considered alone, are seen to follow closely those obtained for synthalin by Bodo and Marks (1928) and for other diamidine derivatives by Broom (1936):

5 mgm. per kilo.	Easily tolerated.	No change.
10 „ „ „	Maximum tolerated dose.	Slight hyperglycaemia.
20 „ „ „	Survived 6 hours.	Hyperglycaemia followed by hypoglycaemia.
15 „ „ „	Survived 4 hours.	Hypoglycaemia.

The onset of hypoglycaemia, which is quite independent of kidney damage, appears, in contrast to the conclusions reached by Broom (1936), to run parallel to hepatic damage and the reduced ability of the liver to deaminize amino acids. Presumably, as with synthalin, it depends first on an initial acceleration of glycogenolysis in the liver. With increasing doses a toxic retardation of the normal process of glyconeogenesis takes place, so that when the glycogen reserves of the liver are exhausted in the hyperglycaemic phase a fatal hypoglycaemia ensues.

In man suffering from malaria, doses up to 150 mgm. undecane diamidine per day can be given orally for three days without affecting the blood sugar level. This does not necessarily exclude the possibility that some slight hepatic injury

is caused by such a dose. Danger to the kidneys is more obvious, however, since an undoubted rise of blood urea and N.P.N. was recorded during the treatment. Considered in conjunction with the toxic symptoms of nausea, vomiting and anorexia, etc., observed clinically, the chemical analyses may be taken to indicate that the dose mentioned is to be regarded as the maximum which can be given with safety. As pointed out by Glyn-Hughes *et al.* (1938) in their clinical studies, undecane diamidine is not recommended for general use in the treatment of malaria.

Incidentally, the results serve to confirm the conclusions reached by Lourie and Yorke (1937), in that it is highly improbable that the parasiticidal action of therapeutic doses is in any way dependent on major metabolic disturbances in the host.

SUMMARY

1. Experiments have been conducted to determine the degree of renal and hepatic injury brought about in the rabbit and in man by administration of various doses of undecane diamidine.

2. In the rabbit, successive small doses (up to a quarter the maximum tolerated dose daily for ten days) intravenously injected are well tolerated without marked effect.

3. A single dose equivalent to about half the M.L.D. produces a rapid rise of blood urea of about 30 mgm. per cent. The blood sugar level is not affected and no albuminuria is found.

4. The maximum tolerated dose (10 mgm. per kilo.) produces a transient hyperglycaemia with marked nitrogen retention. Lethal doses which bring about death in several hours cause a fatal hypoglycaemia with or without an initial hyperglycaemia. The damage to the liver and its deaminizing function runs approximately parallel to the onset of hypoglycaemia.

5. The extent of liver and kidney damage was confirmed by histological examinations.

6. It is pointed out that the toxic action of undecane diamidine on the liver and kidneys broadly resembles that due to synthalin and other alkylene diguanidines.

7. In man, as determined from cases of induced malaria, 150 mgm. undecane diamidine per day administered orally on each of three consecutive days does not affect the blood sugar level, but brings about a definite nitrogen retention. Considered in conjunction with the clinical symptoms of nausea, etc., this is taken to indicate that such a dose is to be regarded as the maximum which can be given with safety to such patients.

ACKNOWLEDGEMENTS.—The author is greatly indebted to Dr. J. L. Dales, who kindly carried out the histological examinations, and to Professor W. Yorke and Dr. E. M. Lourie, for helpful criticism and advice.

REFERENCES

Barr, D. P., and Du Bois, E. F. (1918). Clinical calorimetry. XXVIII : The metabolism in malarial fever. *Arch. Int. Med.*, **21**, 627.

Bischoff, F., Sahyun, M., and Long, M. L. (1929). Guanidine structure and hypoglycaemia. *Jl. Biol. Chem.*, **81**, 325.

Blatherwick, N. R., Sahyun, M., and Hill, E. (1927). Some effects of synthalin on metabolism. *Ibid.*, **75**, 671.

Bodo, R., and Marks, H. P. (1928). The relation of synthalin to carbohydrate metabolism. *Jl. Physiol.*, **65**, 83.

Broom, W. A. (1936). The toxicity and glycaemic properties of a number of amidine and guanidine derivatives. *Jl. Pharm. & Exper. Therap.*, **57**, 81.

Cammidge, P. J. (1927). Discussion on the action of synthalin. *Proc. Roy. Soc. Med.*, **21**, 535.

Conway, E. J. (1933). An absorption apparatus for the micro-determination of certain volatile substances. II : The determination of urea and ammonia in body fluids. *Biochem. Jl.*, **27**, 430.

Fujita, A., and Iwatake, D. (1931). Bestimmung des echten Blutzuckers ohne Hefe. *Biochem. Ztschr.*, **242**, 43.

Glyn-Hughes, F., Lourie, E. M., and Yorke, W. (1938). Studies in chemotherapy. XVII : The action of undecane diamidine in malaria. *Ann. Trop. Med. & Parasitol.*, **32**, 103.

Jancsó, N. von, and Jancsó, H. von (1935). Chemotherapeutische Wirkung und Kohlehydratstoffwechsel: die Heilwirkung von Guanidinderivaten auf die Trypanosomeninfektion. *Ztschr. f. Immunitätsforsch.*, **86**, 1.

King, H., Lourie, E. M., and Yorke, W. (1937). New trypanocidal substances. *Lancet*, **233**, 1360.

Lourie, E. M., and Yorke, W. (1937). Studies in chemotherapy. XVI : The trypanocidal action of synthalin. *Ann. Trop. Med. & Parasitol.*, **31**, 435.

Peters, J. P., and Van Slyke, D. D. (1931). Quantitative clinical chemistry. London.

Schern, K., and Artagaveytia-Allende, R. (1935). La terapia de la tripanosomosis y espirilosis. *Arch. Soc. Biol., Montevideo*, **6**, 244.

——— ——— (1936). Zur glykopriven Therapie und Prophylaxe mit sowohl toxisch als auch atoxisch wirkenden Substanzen bei der experimentellen Trypanosomen- und Treponemen-Infektion. *Ztschr. f. Immunitätsforsch.*, **89**, 21.

STUDIES IN CHEMOTHERAPY*

XIX.—FURTHER REPORT ON NEW TRYPANOCIDAL SUBSTANCES

BY

H. KING

E. M. LOURIE

AND

WARRINGTON YORKE

(*Received for publication June 13th*, 1938)

The discovery that the trypanocidal action of synthalin is a direct one (Lourie and Yorke, 1937) opens a wide field of biological and chemical enquiry. A brief summary of the preliminary results of examination of a considerable number of guanidines, isothioureas, amidines and amines, with alkyl and alkylene chains, was given in a recent paper (King, Lourie and Yorke, 1937). The present communication amplifies the earlier results both chemically and biologically.

The strain of trypanosomes used in the observations here recorded is an old laboratory strain of *Trypanosoma rhodesiense*, which has been maintained since 1923 by passage through mice (Yorke, Adams and Murgatroyd, 1930).

TABLE I

Showing the trypanocidal activity of alkylene diguanidines

General formula $NH_2.C(:NH).NH-(CH_2)_n-NH.C(:NH).NH_2$

Compound	Trypanocidal titre *in vitro*	Toxicity (M.L.D. in mgm. per 20 gm. mouse)	Therapeutic effect in infected mice
Butane diguanidine 2HCl†	1,000,000	4·0	Nil
Pentane diguanidine 2HCl	1,000,000	4·0	,,
Hexane diguanidine 2HCl	8,000,000	1·0	,,
Octane diguanidine 2HCl	64,000,000	0·25	Slight
Decane diguanidine 2HCl (synthalin) ...	256,000,000	0·125-0·25	Considerable
[Diguanylspermine 4HCl‡	256,000	1·0	Nil]
Dodecane diguanidine 2HCl (synthalin B)	256,000,000	0·125	Considerable
Tetradecane diguanidine 2HCl	256,000,000	0·125	,,
Hexadecane diguanidine 2HCl	256,000,000	0·125	Slight
Octadecane diguanidine 2HCl	16/64,000,000	0·25	Nil
Halarsol	256,000,000	0·75	Curative

†We are indebted to Professor D. Ackermann, of the University of Würzburg, for a specimen of this substance and also for a specimen of agmatine.

‡$NH_2.C(:NH).NH.(CH_2)_3.NH.(CH_2)_4.NH.(CH_2)_3.NH.C(:NH).NH_2$.

*This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council.

In this and the following tables :—

Very slight implies that the maximum tolerated doses prolong the life of the animal, but do not entirely clear the blood of trypanosomes.

Slight implies that with maximum tolerated doses the blood of infected mice is cleared of trypanosomes for a number of days, but relapses always occur.

Considerable implies that fractional doses suffice to clear the blood temporarily, and that with maximum tolerated doses, or repeated smaller doses, permanent cures are occasionally obtained.

Curative implies that cures are usually obtained with single easily tolerated doses, or with repeated small doses.

M.L.D. = minimum lethal dose.

Table I summarizes the results obtained with a series of diguanidines, mainly homologues of synthalin. A survey of this table shows that trypanocidal activity rises to a maximum when the chain contains 10 to 16 methylene groups, and then apparently falls away with the higher members. The maximum activity attained *in vitro* is comparable with that observed with the trivalent arsenicals, e.g., halarsol (3-amino-4-hydroxyphenylarsinedichloride). It is noteworthy from the point of view of further exploration in this field that diguanylspermine, which differs from synthalin only in that the chain of 10 methylene groups is broken by two basic imino groups, has but a feeble activity *in vitro*.

The monoguanidines (Table II) also exhibit *in vitro* trypanocidal activity, but of a much lower order than that of the diguanidines. Activity again rises

TABLE II

Showing the trypanocidal activity of alkyl guanidines
General formula $CH_3.(CH_2)_n.NH.C(:NH).NH_2$

Compound	Trypanocidal titre *in vitro*	Toxicity (M.L.D. in mgm. per 20 gm. mouse)	Therapeutic effect in infected mice
[Guanidine carbonate	16,000	10·0	Nil]
[Galegine sulphate (isopentenyl-guanidine)*	256,000	0·5	„]
[Hydroxyhydrogalegine sulphate ... ... (Hydroxyisoamylguanidine)†	16,000	>10·0	„]
Nonylguanidine HI	64,000	1·0	„
Dodecylguanidine HCl	4,000,000	0·25	„
Tetradecylguanidine HCl	4,000,000	0·5	„
Hexadecylguanidine HCl	4,000,000	1·0	„
Octadecylguanidine HCl	500,000	8·0	„

*$(CH_3)_2.C : CH.CH_2.NH.C(:NH).NH_2$.

†$(CH_3)_2.C(OH).CH_2.CH_2.NH.C(:NH).NH_2$.

to a maximum as the unbranched homologous series is ascended, and then falls off. In galegine, which has a branched unsaturated carbon chain, the unsaturation seems to enhance the activity ; on saturation of the ethylene linkage by addition of the elements of water, as in hydroxyhydrogalegine, activity falls to a low level.

Certain well-known guanidines containing amino or carboxyl groups, and other simple cyclic guanidines of the type of creatinine, exhibit very little *in vitro* trypanocidal activity (Table III).

TABLE III

Showing the trypanocidal activity of certain well-known guanidines

Compound	Formula	Trypanocidal titre *in vitro*	Toxicity (M.L.D. in mgm. per 20 gm. mouse)	Therapeutic effect in infected mice
Arginine ...	$CO_2H.CHNH_2.(CH_2)_3.NH.C(:NH).NH_2$	1,000	>10·0	Nil
Agmatine ...	$CH_2NH_2.(CH_2)_3.NH.C(:NH).NH_2$	64,000	>16·0	,,
Glycocyamidine	$CO.CH_2.NH.C(:NH).NH$	1,000	>10·0	,,
Alacreatinine ...	$CO.CH(CH_3).NH.C(:NH).NH$	<1,000	>10·0	,,
Creatinine ...	$CO.CH_2.N(CH_3).C(:NH).NH$	1,000	>10·0	,,

The results recorded in the foregoing tables demonstrate that maximum trypanocidal activity is shown by a plurality of guanidine groups attached to an inert carbon structure, provided that the molecule has a certain optimum size. The guanidine group $NH:C(NH_2)NH$- is, however, not essential for trypanocidal activity, since the connecting imino-link, -NH-, between the guanyl

TABLE IV

Showing the trypanocidal activity of alkylene di-isothioureas
General formula $NH_2.C(:NH).S-(CH_2)_n-S.C(:NH).NH_2$

Compound	Trypanocidal titre *in vitro*	Toxicity (M.L.D. in mgm. per 20 gm. mouse)	Therapeutic effect in infected mice
Ethane di-isothiourea 2HBr	2,000,000	4·0	Nil
Propane di-isothiourea 2HBr	8,000,000	4·0	Slight
Hexane di-isothiourea 2HBr	8,000,000	4·0	Considerable
Decane di-isothiourea 2HBr	8/16,000,000	2·0	Nil
Undecane di-isothiourea 2HBr	8/16,000,000	2·0	,,
Dodecane di-isothiourea 2HBr	8/16,000,000	1·0	,,
Tetradecane di-isothiourea 2HBr ...	16/64,000,000	1·0	,,
Hexadecane di-isothiourea 2HBr	64,000,000	0·5	,,

group NH : $C(NH_2)$- and the carbon chain, can be replaced by a sulphur atom, e.g., NH : $C(NH_2)$-S-$(CH_2)_n$ . . . , with considerable retention of activity (Table IV). In this series of di-isothioureas, trypanocidal activity again rises as the series is ascended.

Since trypanocidal activity was retained by this simple modification of the mode of attachment of the guanyl grouping to the carbon chain or structure, it was naturally of interest to determine the effect of attaching the guanyl grouping directly to the carbon chain without the intervention of other atoms. The substances so obtained are amidines and contain the grouping NH : $C(NH_2).(CH_2)_n$ . . . The results observed with a series of mono- and di-amidines are recorded in Table V.

TABLE V

Showing the trypanocidal activity of alkyl amidines and alkylene diamidines
General formulae $CH_3.(CH_2)n.C(: NH).NH_2$ and $NH_2.C(: NH)–(CH_2)_n–C(: NH).NH_2$

Compound	Trypanocidal titre *in vitro*	Toxicity (M.L.D. in mgm. per 20 gm. mouse)	Therapeutic effect in infected mice
Palmitamidine HCl	4,000,000	1·0	Nil
Stearamidine HCl	4/16,000,000	2·0	,,
Octane diamidine 2HCl	8,000,000	1·0	Very slight
Nonane diamidine 2HCl	16,000,000	1·0	Considerable
Decane diamidine 2HCl	64,000,000	0·5	Curative
Undecane diamidine 2HCl*	256,000,000	0·5	,,
Dodecane diamidine 2HCl	64,000,000	0·5	,,
Tridecane diamidine 2HCl	16/64,000,000	0·5	,,
Tetradecane diamidine 2HCl	16/64,000,000	0·5	,,
Hexadecane diamidine 2HCl	4/16,000,000	0·5	Slight

*We are indebted to Dr. F. L. Pyman, F.R.S., of Boots Pure Drug Company, for a specimen of this compound.

In this series of homologous diamidines, trypanocidal activity again rises to a maximum as the series is ascended, and then gradually falls off, the most effective member being undecane-1 : 11-diamidine, which has a curative action in infected mice and rabbits.

It may be remarked in passing that Kawai, Hosono, Shikinami and Yonechi (1931), in an attempt to prepare hypoglycaemic substances less toxic than synthalin, also synthesized a series of di-isothioureas and diamidines.

It is possible to analyse the origin of the trypanocidal activity of the foregoing compounds a step farther, for when the guanyl grouping NH : $C(NH_2)$- is replaced by the amino group NH_2- there is still a noticeable trypanocidal

effect *in vitro* (Table VI), but this is definitely inferior to that observed with the diguanidines and diamidines.

TABLE VI

Showing the trypanocidal activity of alkyl amines and alkylene diamines
General formulae $CH_3.(CH_2)_n.NH_2$ and $NH_2.(CH_2)_n.NH_2$

Compound	Trypanocidal titre *in vitro*	Toxicity (M.L.D. in mgm. per 20 gm. mouse)	Therapeutic effect in infected mice
Nonylamine HCl	500,000	4·0	Nil
Tetradecylamine HCl	4,000,000	2·0	,,
Hexadecylamine HCl	4/8,000,000	2·0	,,
Octadecylamine HCl	2,000,000	4·0	,,
Octane diamine 2HCl	250,000	8·0	Nil
Undecane diamine 2HCl	1,000,000	8·0	,,
Tetradecane diamine 2HCl...	1/4,000,000*	2·0	,,
Hexadecane diamine 2HCl	1/4,000,000	0·5	Slight
Octadecane diamine 2HCl	16/64,000,000	0·5	Nil

*In a preliminary paper this figure was incorrectly given as 32,000,000.

If the trypanocidal action of the diamines is due to the same mechanism as operates in the case of the diguanidines, di-isothioureas and diamidines,

TABLE VII

Showing the trypanocidal activity of quaternary ammonium compounds
General formulae $CH_3.(CH_2)_n.\overset{+}{N}(CH_3)_3$ and $\overset{+}{N}(CH_3)_3.(CH_2)_n.\overset{+}{N}(CH_3)_3$

Compound	Trypanocidal titre *in vitro*	Toxicity (M.L.D. in mgm. per 20 gm. mouse)	Therapeutic effect in infected mice
Trimethylnonylammonium chloride ...	64,000	2·0	Nil
Trimethylnonylammonium iodide	64,000	2·0	,,
Trimethyloctadecylammonium chloride ...	256,000	1·0	,,
Hexamethyloctamethylene *bis* ammonium chloride	1,000	0·25	Nil
Hexamethyltetradecamethylene *bis* ammonium chloride	16,000	0·5	,,

then the only structural feature common to this group of substances is the possession of a central inert carbon chain with terminal polar groups of a strongly basic nature. On this view of the structural requirements it was important to investigate the effect of conversion of the terminal amino groups into the still more strongly polar quaternary ammonium salts by methylation (Table VII).

An examination of these results shows that quaternary ammonium compounds have very little activity. It is doubtless significant that their chemical structure precludes them from acting other than as strong ions—in other words, they cannot give free bases by hydrolysis of their salts. This naturally suggests that the trypanocidal activity of the amidines, guanidines, isothioureas and amines, which are all strong bases forming neutral salts, is a property of the free bases liberated by partial hydrolysis.

The effect of substitution in the guanyl group $NH : C(NH_2)$- by alkyl or other type of radicle opens up a wide field for exploration. Glyoxalines and dihydroglyoxalines (iminazolines) may be looked upon as cyclic disubstituted amidines, whilst 2-thioglyoxalines may be regarded as cyclic disubstituted isothioureas. A few representatives of these types have been made and examined (Table VIII). The results show that a considerable activity is retained in certain of these cyclic compounds. The last compound in the list, decane-1 : 10-dipiperidine, may be regarded as a cyclic tetrasubstituted diamine.

TABLE VIII

Showing the trypanocidal activity of certain glyoxalines, dihydroglyoxalines and thioglyoxalines (cyclic disubstituted amidines and isothioureas)

Compound	Trypanocidal titre *in vitro*	Toxicity (M.L.D. in mgm. per 20 gm. mouse)	Therapeutic effect in infected mice
2-Amyldihydroglyoxaline HCl	16,000	2·0	Nil
„ acid oxalate ...	16,000	2·0	„
2-Octyldihydroglyoxaline HCl	256,000	2·0	„
„ acid oxalate ...	256,000	2·0	„
2-Hexylglyoxaline acid oxalate	256,000	2·0	„
2-Pentadecyl-4-methylglyoxaline HCl ...	4,000,000	4·0	„
Decane-1 : 10-di-(4′-methyl-2′-glyoxaline) 2HCl	256,000,000	0·5	Considerable
Decane-1 : 10-di-(4′-methyl-2′-thioglyoxaline) 2HBr	64,000	>8·0	Nil
Decane-1 : 10-di-(4′-methyl-2′-thioglyoxaline) diacid oxalate	64,000	>8·0	„
Decane-1 : 10-dipiperidine 2HCl	1,000,000	4·0	„

Examination of the data presented in Tables I to VIII shows that, provided one knows the trypanocidal titre *in vitro* of a compound, and its toxicity for mice, one can form a shrewd opinion whether or not the compound will have any therapeutic action in infected mice. Among the compounds examined those possessing the greatest trypanocidal action *in vitro* are the decane, dodecane, tetradecane and hexadecane diguanidines (Table I), and the decane, undecane and dodecane diamidines (Table V). Of the former group, decane diguanidine (synthalin) is the least toxic, the minimum lethal dose per 20 gm. mouse being about 0·25 mgm., although 0·15 mgm. frequently produces toxic signs; the maximum well-tolerated dose is about 0·075 to 0·1 mgm. The toxicity of each

TABLE IX

Comparing the therapeutic action of synthalin and undecane diamidine in mice infected with *T. rhodesiense*

	Dose (mgm. per 20 gm. mouse)	Synthalin		Undecane diamidine	
		No. of mice	Result	No. of mice	Result
Single doses	0·25	5	1C(T) 4P	10	8C 2R
	0·15	10	1C 2P 7R(T)	10	4C 6R
	0·075	10	10R	—	—
	0·05	5	5R	6	6R
	0·025	5	5NN	5	5R
Repeated doses	0·25 on each of 5 consecutive days... ...	—	—	10	10C
	0·15 „ „ „ „	—	—	10	10C
	0·075 on 1st day and 0·05 on 4 following days	10	2C 8R(T)	10	8C 2R
	0·05 at 3- or 4-day intervals for 5 doses ...	10	10R	10	8C 2R
	0·05 at 3-day intervals for 2 doses	5	5R	5	3C 2R

C = Cured. No relapse in an observation period of 2 to 3 months.
P = Poisoned. The animal was killed by the drug.
(T) = Toxic. The animal exhibited toxic signs, but recovered.
R = Relapsed. The blood became negative, but trypanosomes reappeared after an interval.
NN = Not negative. Trypanosomes did not disappear from the blood.

of the three compounds of the later group is about the same, and is definitely less than that of synthalin ; the minimum lethal dose per 20 gm. mouse is about 0·5 mgm., and the maximum well-tolerated dose is at least 0·25 mgm. As the undecane member is of rather greater trypanocidal activity than either of the other two, it was decided to compare its therapeutic action with that of synthalin.

The results of these experiments in mice, which are summarized in Table IX, show clearly the pronounced superiority of the therapeutic action of undecane diamidine. With a single well-tolerated dose (0·25 mgm. per 20 gm. mouse) no less than 80 per cent. permanent cures were obtained, and with a dose of about half this size (0·15 mgm.) permanent cures were obtained in 4 of 10 mice. Synthalin given in the larger of these doses (0·25 mgm.) killed 4 of 5 mice, and with the smaller dose, notwithstanding the fact that it produced toxic signs, only 1 cure was obtained in the 10 mice treated. That the superiority of undecane diamidine is not entirely due to its lower toxicity is further shown by the fact that the very small dose of 0·025 mgm. per 20 gm. mouse sufficed temporarily to clear the blood of all 5 mice treated with undecane diamidine, but of none of those treated with synthalin. The various methods of treatment by repeated fractional doses bring out equally the therapeutic superiority of undecane diamidine ; and it will be seen that with repeated doses of suitable size approximately 100 per cent. permanent cures may be obtained.

Attempts to cure infected rabbits with synthalin were unsuccessful. Temporary improvement resulted from a course of the largest tolerated doses, but relapses soon followed. The results obtained with undecane diamidine were much more encouraging, and rabbits in a late stage of the infection, with pronounced lesions of the skin, mucous membranes and genitalia, responded readily to treatment of this compound. Preliminary results showed that, although a single dose of 10 mgm. of the compound per kilo. of body weight was often tolerated by normal rabbits, it usually killed infected animals ; whereas doses of 5 mgm. or less per kilo. produced no ill effect. Accordingly, a series of 32 rabbits, inoculated with *T. rhodesiense* about three weeks previously, and all showing pronounced signs of the disease, were treated with various courses of the drug, with the following results :

Details of Rabbits Treated by Intravenous Injection of Undecane Diamidine

A. *5 mgm. per kilo. on each of 5 consecutive days*

169d. Became normal ; no relapse in 6 months. Cured.

169e. Became normal ; relapsed 39 days after last dose. Treatment repeated ; no relapse in 6 months. Cured.

169f. Became normal ; no relapse in 6 months. Cured.

336a. Became normal ; no relapse in 6 months. Cured.

336b. Became normal, but relapsed 43 days after last dose. Treatment repeated ; no relapse in 4 months. Cured.

336c. Improved, but died 2 days after last dose from unknown cause—probably toxic effects of drug.

B. 5 *mgm. per kilo. at* 3- *and* 4-*day intervals for* 5 *doses*

991e. Became normal, but relapsed 25 days after last dose. Treatment repeated ; no relapse in 6 months. Cured.

991f. Became normal ; no relapse in 6 months. Cured.

34a. Became normal, but relapsed 28 days after last dose. Treatment repeated ; no relapse in 6 months. Cured.

34b. Became normal ; no relapse in 6 months. Cured.

34d. Became normal, but relapsed 54 days after last dose. Treatment repeated ; no relapse in 6 months. Cured.

C. 2·5 *mgm. per kilo. on each of* 10 *consecutive days.*

170b. Became normal ; no relapse in 3 months. Cured.

170c. Became normal, but relapsed 7 weeks after last dose. Treatment repeated ; no relapse in 5 months. Cured.

170f. Became normal ; no relapse in 6 months. Cured.

170d. Became normal ; no relapse in 6 months. Cured.

444b. Became normal ; no relapse in 4 months. Cured.

D. 2·5 *mgm. per kilo. on each of* 5 *consecutive days*

76b. Became normal ; no relapse in 6 months. Cured.

76c. Became normal ; no relapse in 6 months. Cured.

76d. Became normal, but relapsed 4 weeks after last dose. Treatment repeated ; no relapse in 6 months. Cured.

77a. Became normal ; no relapse in 6 months. Cured.

170a. Signs disappeared, but immediately after 2nd dose animal had a convulsion and became paralysed in hind legs. The last 3 doses produced no untoward result, but animal died with paralysis and incontinence 12 days after last dose. Death can hardly have been due to the toxic action of the drug *per se* ; possibly something in the nature of a Herxheimer reaction had occurred.

444a. Became normal, but relapsed 8 weeks after last dose. Treatment repeated ; no relapse in 3 months. Cured.

E. 2·5 *mgm. per kilo. at* 3- *and* 4-*day intervals for* 5 *doses*

991d. Became normal ; no relapse in 6 months. Cured.

34c. Treatment without effect. Treatment repeated, again without effect. This was followed by 2·5 mgm. daily for 16 consecutive days, and subsequently by 5 mgm. daily for a similar period. No effect, the animal eventually dying of the disease. Shortly before death the trypanosomes were transferred to mice and found to be resistant to the drug.

34e. Became normal, but relapsed 1 week after last dose. Treatment repeated ; no relapse in 6 months. Cured.

77b. Became normal, but relapsed 3 weeks after last dose. Treatment repeated ; relapsed 10 days after last dose. Treated again with 5 mgm. at 3- and 4-day intervals for 5 doses ; relapsed 6 weeks after last dose. Treated again with 5 mgm. daily for 10 consecutive days ; no relapse in 4 months. Cured.

77c. Became normal, but relapsed 4 weeks after last dose. Treatment repeated ; no relapse in 6 months. Cured.

F. 1·25 *mgm. per kilo. on each of* 10 *consecutive days*

76e. Became normal, but relapsed 2 weeks after last dose. Treatment repeated ; no relapse in 6 months. Cured.

76f. Became normal, but relapsed 2 weeks after last dose. Treatment repeated ; relapsed 3 weeks after last dose. Treated with 2·5 mgm. daily for 10 days ; no effect. Then given 5 mgm. daily for 6 days without effect, the animal dying shortly afterwards with paralysis.

77e. Became normal ; no relapse in 6 months. Cured.

77f. Became normal, but relapsed 1 week after last dose. Treatment repeated ; relapsed 10 days after last dose. Treatment repeated ; no effect. Treated with 2·5 mgm. daily for 10 consecutive days ; no effect. Then given 5 mgm. daily for 6 days without effect, the animal dying.

77d. Became normal ; no relapse in 6 months. Cured.

TABLE X

Summarizing the results of treating rabbits with various courses of n. undecane-1 : 11-diamidine

Treatment	No. of rabbits treated	Number cured			Remarks
		By 1st course	By 2nd course	By both courses	
A. 5 mgm. per kilo. on each of 5 consecutive days	6	3	2	5	1 died (poisoned ?) shortly after completion of 1st course
B. 5 mgm. per kilo. at 3- and 4-day intervals for 5 doses	5	2	3	5	
C. 2·5 mgm. per kilo. on each of 10 consecutive days	5	4	1	5	
D. 2·5 mgm. per kilo. on each of 5 consecutive days	6	3	2	5	1 died from unknown cause shortly after completion of 1st course
E. 2·5 mgm. per kilo. at 3- and 4-day intervals for 5 doses	5	1	2	3	1 resisted treatment from beginning; 1 relapsed after 2 courses and was eventually cured by a larger course
F. 1·25 mgm. per kilo. on each of 10 consecutive days	5	2	1	3	2 relapsed after the two courses, and were then treated unsuccessfully with larger courses

It will be seen from the details given above and from the summary in Table X that, of the 22 rabbits subjected to the more intensive courses of treatment (A-D), one (336c) died shortly after the end of the most intense course of treatment (A) with signs suggesting that death had probably resulted from the toxic effects of the drug; and another (170a) developed convulsions and paralysis immediately after the second injection of Course D, but as three further injections were given without any apparent ill effect, and as the animal survived for 12 days afterwards, death in this case can hardly be ascribed to the toxic action of the drug *per se*, and may possibly have been due to something

in the nature of a Herxheimer reaction. Of the remaining 20 animals, all were cured, either by the first or the second course of treatment. The criterion of cure in these cases was no sign of relapse during a period of 5–6 months after termination of treatment (in 5 cases the observation is at the time of writing, only 3–4 months). If we confine ourselves to the 20 animals which survived, we find that 12 (60 per cent.) were cured by the first course of treatment, and that of the 8 which relapsed all (100 per cent.) were cured by the second course of treatment. Why only 60 per cent. of the animals should have been cured by the first course of treatment, whilst 100 per cent. of those which relapsed should have been cured by an exactly similar second course of treatment, is a question of some interest for which we are unable to offer an adequate explanation at the present time. At first sight one would have expected the first course of treatment to have given a larger proportion of successes than that obtained by subjecting the animals which had relapsed to an exactly similar second course of treatment. The phenomenon would at least afford strong evidence that the initial course of treatment had not to any appreciable extent enhanced the resistance of the parasite to the drug; but this fact alone would not suffice to explain why the second course gave a larger proportion of cures than the first—exactly similar—course of treatment. It may be that the first course had, instead of increasing the resistance of the parasites, actually made them more sensitive to the drug; but for this hypothesis we have, as yet, found no support. Or the explanation may possibly lie in the fact that the condition of the animals at the beginning of the second course of treatment was much better than it was at the commencement of the initial course of treatment. As already mentioned, the rabbits were not treated until about three weeks after infection, when they were exhibiting pronounced signs of the disease and were obviously ill. The relapses were, however, treated as soon as they were discovered, when the animals showed little else than some localized oedemas, which on puncture revealed the presence of trypanosomes. It does not seem unreasonable to expect, all other factors being equal, that a better response will be obtained from animals treated when in good condition than when in an obviously bad state.

Of the 10 animals which received less intensive courses of treatment (E and F), 3 were cured by the first course and 3 by the second course. Here again we see the same phenomenon, viz., that a higher proportion of animals were cured by the second course of treatment than by the first. Thirty per cent. of the 10 animals which were given the first course treatment were permanently cured, whereas 50 per cent. of the 6 animals which relapsed after the first course were cured by the second course (the animal (34c) which failed to respond to the first course (E) is omitted from this calculation).

These less intensive courses are clearly not nearly so satisfactory as the more intensive courses, for the following reasons:

1. Two such courses cured only 60 per cent. of the animals.

2. One animal (34c) resisted the treatment from the very beginning, and subsequent courses of more intense treatment had no other result than to make the infection completely drug-resistant.

3. Two other animals (76f and 77f), which had relapsed after each of the first two courses of treatment, were subsequently treated unsuccessfully with more intensive courses: the infections in both animals became eventually completely drug-resistant.

4. One animal (77b) which had relapsed after each of the first two courses of treatment was subsequently cured by more intense treatment.

It follows, therefore, that the less intense courses of treatment are unsatisfactory, not only because they fail to cure all the animals treated, but because there is a considerable probability that those which relapse cannot be cured by the more intense forms of treatment, as the infections have developed drug-resistance. Drug-resistance to undecane diamidine will form the subject of a later paper of this series.

The results of these experiments seem to justify the general conclusion that by the administration of suitable courses of undecane diamidine 100 per cent. of rabbits in an advanced stage of infection with *T. rhodesiense* can be cured.

Effect of Undecane Diamidine on *T. congolense*

A number of experiments were performed in order to determine whether this compound has any action on *T. congolense* infections in mice. The results can be summarized briefly as follows:

5 infected mice were given a single maximum well-tolerated dose of 0·25 mgm. per 20 gm.; the only result was a slight prolongation of the infection.

5 infected mice were given 0·25 mgm. daily for 5 consecutive days; all the animals became negative, but all relapsed in about 10 days.

5 mice were given 0·25 mgm. per 20 gm. 30 minutes after inoculation and on each of the 9 following days; only 1 became infected.

It appears, therefore, that the action of undecane diamidine on *T. congolense* infections is much less than on *T. rhodesiense* infections. A single maximum well-tolerated dose of the drug has practically no therapeutic effect on *T. congolense* infections in mice; a series of maximum doses on each of 5 consecutive days has a definite effect, in that the blood becomes negative, but relapses occur in about 10 days; whilst a series of maximum doses commencing on the day of inoculation has a pronounced prophylactic action, in that the majority of the infections fail to develop. Browning (1938) has shown that synthalin exerts a very slight therapeutic action on mice infected with *T. congolense*.

Effect of Undecane Diamidine on *T. cruzi*

A series of experiments similar to the above were performed on mice infected with *T. cruzi.* The drug has apparently not the slightest therapeutic or prophylactic effect. This observation corresponds with that of Schern and Artagaveytia-Allende (1936), who found that synthalin had no action on *T. cruzi* infections.

Effect of Undecane Diamidine on Spirochaetes and Spirilla

Similar experiments conducted on mice infected with *Spirochaeta recurrentis* or *Spirillum minus* gave entirely negative results, the drug exhibiting no therapeutic or prophylactic action.

This is somewhat surprising, as Schern and Artagaveytia-Allende found that synthalin had a definite action on *Treponema hispanicum* infections in mice ; on our strain of *Spirochaeta recurrentis*, however, synthalin had not the slightest action.

Effect of Undecane Diamidine on *Plasmodium vivax*

Undecane diamidine has a definite action on human simple tertian malaria. Details of our observations on this subject are found in a previous paper of this series (Glyn-Hughes, Lourie and Yorke, 1938).

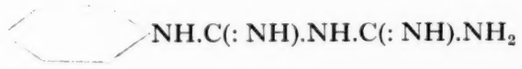
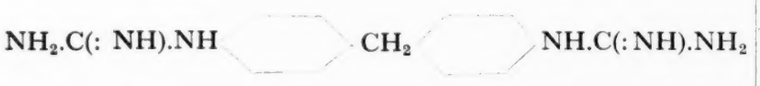
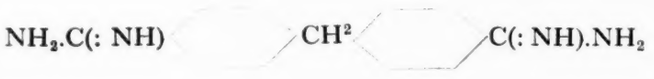
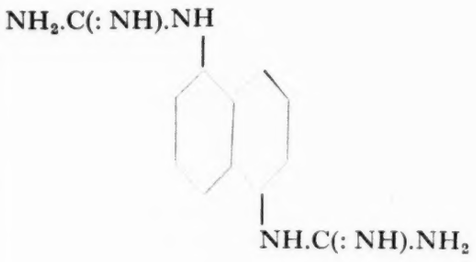
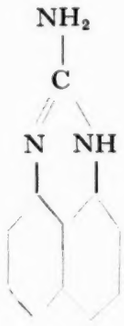
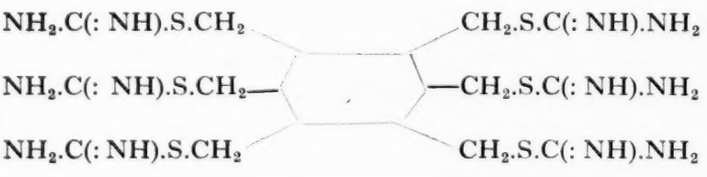
Aromatic Amidine and Guanidine Trypanocidal Substances

In all the compounds so far considered the active polar groupings have been carried by a long inert carbon chain of methylene groups, maximum activity manifesting itself in the region of 10 to 14 methylene groups. It seemed possible that this carbon chain merely served as a carrier of the active groupings, and might be replaced by an inert aromatic structure of approximately the same molecular weight. The results of examination of a number of such compounds is shown in Table XI, from which it is seen that certain of them exhibit considerable trypanocidal activity *in vitro*, and that three, viz., pp′-diguanidino-diphenyl-methane, pp′-diamidino-diphenyl-methane, and 2 : 7-naphthalene diamidine, actually cure infected mice.

In Table XII a few miscellaneous compounds are collected. The higher mono-guanidines, mono-amidines, etc., have the attributes of typical soaps, like sodium stearate, and it seemed a possibility that their trypanocidal activity was due in part to this property. A few soaps and surface-tension lowering substances have therefore been examined, but their trypanocidal activity is of such a low order as to preclude any interpretation of the trypanocidal action of mono-alkylguanidines or mono-alkylamidines in terms of their surface-tension lowering properties.

TABLE XI

Showing the trypanocidal activity of certain aromatic amidines and guanidines

Compound	Formula	Trypanocidal titre *in vitro*	Toxicity (M.L.D. in mgm. per 20 gm. mouse)	Therapeutic effect in infected mice
Phenylguanidine		16,000	8·0	Nil
Phenyldiguanide		16,000	>8·0	"
pp′-diguanidinodiphenylmethane		16,000,000	2·0	Curative
pp′-diamidinodiphenylmethane*		4/16,000,000	1·0-2·0	"
1:5-diguanidinonaphthalene		64/256,000	0·5	Nil
2 : 7-naphthalene diamidine		4/16,000,000	1·0-2·0	Curative
Aminoperimidine		1,000,000	1·0	Nil
Hexaisothiourea methyl benzene†		1/4,000,000	0·25	Nil

*Prior to the preparation of this compound we were aware of its trypanocidal activity from a private communication by Dr. A. J. Ewins, of Messrs. May and Baker, Ltd.

†For this substance we are indebted to Professor H. J. Backer of the University of Groningen.

TABLE XII

Showing the trypanocidal activity of a few miscellaneous compounds including some of a soapy nature

Compound	Trypanocidal titre *in vitro*	Toxicity (M.L.D. in mgm. per 20 gm. mouse)	Therapeutic effect in infected mice
Sebacic acid *bis* iminoether 2HCl	1,000	8·0	Nil
Sodium stearate	4,000	8·0	„
„ palmitate	4,000	8·0	„
„ laurate	4,000	8·0	„
„ ricinoleate	4,000	8·0	„
Hexylresorcinol	8,000	2·0	„
Sodium decane-1 : 10-disulphonate ...	1,000	>8·0	„
Aminoguanidine sulphate	16,000	>8·0	„
Glucoseguanylhydrazone sulphate	<16,000	>8·0	„

SUMMARY

1. The investigation described in this paper was instigated by the discovery that synthalin has a powerful direct trypanocidal action.

2. A considerable number of guanidines, isothioureas, amidines and amines, with alkyl and alkylene chains, were prepared and examined for trypanocidal activity.

3. It was found that certain of the diamidines exhibit a powerful trypanocidal action *in vitro*, and that with the most active member of the series, viz., n. undecane-1 : 11-diamidine, it is possible to produce permanent cures in approximately 100 per cent. of mice and rabbits infected with our laboratory strain of *T. rhodesiense*.

4. Undecane diamidine had little curative action on mice infected with *T. congolense*, but when given in large doses on the day of inoculation and on the following nine days it exhibited a pronounced prophylactic action.

5. This compound had no action on *T. cruzi* infections in mice, or on mice infected with *Spirochaeta recurrentis* or *Spirillum minus*.

6. It has a definite action on human simple tertian malaria, causing the parasites to disappear from the peripheral blood and the febrile paroxysms to cease.

7. The structural feature common to all the compounds referred to above is the possession of a central inert carbon chain with terminal polar groups of strongly basic nature.

8. As it seemed possible that this carbon chain merely served as a carrier of the active groupings, and might be replaced by an inert aromatic structure of approximately the same molecular weight, a number of aromatic amidine and guanidine compounds were prepared.

9. Several of these aromatic compounds were found to exhibit pronounced trypanocidal action *in vitro*, and with three of them, viz., pp′-diguanidino-diphenyl-methane, pp′-diamidino-diphenyl-methane, and 2 : 7-naphthalene diamidine, it was found possible to cure mice infected with *T. rhodesiense*.

10. This work opens up a new field in the search for substances of therapeutic value against trypanosomal and malarial infections.

REFERENCES

BROWNING, P. (1938). The chemotherapeutic action of synthalin in experimental infections with *T. brucei* and *T. congolense*. *Jl. Path. & Bact.*, **46**, 323.

GLYN-HUGHES, F., LOURIE, E. M., and YORKE, W. (1938). Studies in chemotherapy. XVII : The action of undecane diamidine in malaria. *Ann. Trop. Med. & Parasitol.*, **32**, 103.

KAWAI, S., HOSONO, T., SHIKINAMI, Y., and YONECHI, S. (1931). Researches on hypoglycemia producing substances. II : Pseudothiourea, amidine and urea derivatives. *Sci. Pap. Inst. Phys. & Chem. Res., Tokyo*, **16**, 9.

KING, H., LOURIE, E. M., and YORKE, W. (1937). New trypanocidal substances. *Lancet*, 233, 1360.

LOURIE, E. M., and YORKE, W. (1937). Studies in chemotherapy. XVI : The trypanocidal action of synthalin. *Ann. Trop. Med. & Parasitol.*, **31**, 435.

SCHERN, K., and ARTAGAVEYTIA-ALLENDE, R. (1936). Zur glykopriven Therapie und Prophylaxe mit sowohl toxisch als auch atoxisch wirkenden Substanzen bei der experimentellen Trypanosomen- und Treponemen-Infektion. *Ztschr. f. Immunitätsforsch.*, **89**, 21.

YORKE, W., ADAMS, A. R. D., and MURGATROYD, F. (1930). Studies in chemotherapy. II : The action *in vitro* of normal human serum on the pathogenic trypanosomes, and its significance. *Ann. Trop. Med. & Parasitol.*, **24**, 115.

SOME OBSERVATIONS ON GUINEA-WORM LARVAE

BY
T. SOUTHWELL
AND
A. KIRSHNER

(*From the Department of Parasitology, Liverpool School of Tropical Medicine*)

(*Received for publication June 1st*, 1938)

The supply of guinea-worm larvae on which our observations are based was obtained from an Indian patient admitted to the Tropical Ward of the Liverpool Royal Infirmary. A small ulcer was present over the left lateral malleolus, from which larvae could be obtained in large numbers. The adult worm was not palpable anywhere under the skin.

In February, when we made our collection, cyclops were difficult to obtain from ponds near Liverpool. The weather was cold, and we succeeded in procuring only a comparatively small number. The collection comprised several species, including, amongst others, *C. bisetosus* (= *C. bicuspidatus*) and *C. vernalis* (= *C. lucidulus* = *C. robustus*). It was in the latter species, namely *C. vernalis*, that guinea-worm larvae were mostly found.

As far as we are aware, guinea-worm larvae have not before been recorded as developing in *C. vernalis*. It may be that the incomplete development which we observed in this species is due to the fact that it is not a normal intermediate host for *Dracunculus medinensis*. In this connection it is interesting to note that in other species of cyclops a partial development of guinea-worm larvae takes place but is never complete—as, for instance, in *C. prasinus*.* It was noted that there was present a considerable number of *Daphnia* sp., but these crustacea never attempted to swallow guinea-worm larvae. On numerous occasions when larvae passed near the mouth of *Daphnia* they were kicked back violently and very often damaged in the process.

Viability of Larvae in Pond- and Tap-Water

It was noted that larvae collected from the ulcer in sterile tap-water died in about 6 hours.

In order to keep them alive, it was necessary to transfer them immediately to pond-water. In ordinary pond-water kept at a temperature of 17° C., the larvae rarely lived more than 9 days. Almost immediately after being placed in the water all the larvae sank to the bottom. For the first 4 days they appeared to be very active in their movements, though a small number died each day. On the 5th day rapidity of movement lessened, and by the 8th day a large

*For a complete list of synonyms of *C. prasinus*, see Gurney (1933), p. 87.

majority of them were dead. Those that remained alive exhibited very sluggish movements. At the end of the 9th day all the larvae appeared to be dead. Practically the same results were noted with pond-water from different sources.

Certain observers state that in the tropics the larvae remain viable in water for two weeks.

Mode of Entry of Larvae into Cyclops

Early observers stated quite definitely that the larvae penetrated the armour of cyclops by attacking it between the joints of the ventral plates, and that they did not enter the body cavity through the alimentary canal after being swallowed. This opinion, however, has proved to be untrue.

In our experiments, some 50 cyclops in all were placed, on different occasions, with guinea-worm larvae, and in no instance were the larvae ever seen to attempt penetration into the body, nor were they ever found in the haemocoele within a few hours of being swallowed. The larvae are known to be actively ingested by the crustacean After placing larvae and certain species of cyclops together, the larvae were on numerous occasions found in the stomach of cyclops a few hours later. Moorthy (1935) has shown us a photograph of a cyclops actually swallowing a larva.

Whilst examining infected cyclops, it was found a simple matter to dissect out the stomach and to see the contained larvae, where present. The procedure is as follows : A cyclops is placed on a slide in a drop of water, and, with the aid of a binocular microscope, the head is fixed with a very fine needle. The blunt side of a Hagedorn needle is placed flat on the tail, which is gently pulled away from the body. By this method the whole of the alimentary canal is liberated from the body.

A number of larvae were placed in a Petri dish with five cyclops. After 10 minutes no larvae remained—all had been ingested. Dissection of the cyclops by the method noted above showed that the stomachs contained respectively 5, 10, 8, 3 and 6 larvae.

In another instance one cyclops was seen to ingest 20 larvae in about 15 minutes, and this number was confirmed by dissection.

It has been stated that the lashing movements of the larvae attract the attention of the cyclops in the same way that fish are attracted by the movements of a fly. This, however, is not correct, for when dead larvae were placed with cyclops they were seen to be greedily ingested.

In a large number of cases most of the larvae had escaped from the gut into the haemocoele within 24 hours. This migration of the larvae through the stomach wall was observed in many cases. Moorthy (1938) notes that in his experiments the larvae reach the body cavity in from one to six hours after being swallowed.

It was noted that the larvae which had just escaped from the stomach into the haemocoele appeared darker than those taken from the guinea-worm. A com-

parison showed that the intestines of those which had previously been in the stomach of cyclops contained a granular material, while the intestine of those obtained directly from the guinea-worm were perfectly clear and empty. It appears, therefore, that the larvae feed in the stomach of cyclops before penetrating into the haemocoele. As the larvae develop further in the body cavity, the granular material tends to become more dense.

Number of Larvae Lethal to Cyclops

Some observers have noted that the presence of more than seven larvae usually results in the death of cyclops. In our specimens a dissection of a number showed that quite a few contained 7, 8 and 9 larvae. One cyclops was found to contain 15 larvae in its haemocoele, another 12 larvae, and a third 10 larvae. In all cases the cyclops were alive and active.

Manson (1895, p. 1350) states that in his experiments he ' found that nearly every one of them [cyclops] had some 10 or 20 guinea-worm embryos coiled up and wriggling about in their bodies.'

Development of Larvae in Cyclops

This was not followed completely. The average measurement of the larvae obtained from the adult worm was about 650μ in length and 21μ in breadth. The oesophagus, though varying from 145μ to 170μ, usually measured 150μ in length ; the intestine averaged 230μ ; and the tail about 270μ in length. The nerve cord was generally situated about 80μ from the anterior extremity.

No development of the larvae in the haemocoele of cyclops could be observed up to 22 days after infection, apart from an increasing rugosity of the skin and the presence of granular material in the intestine (noted above).

Daily measurements showed no appreciable alterations in size or breadth, and no development of internal organs.

In the 4th week a small but definite constriction was noticed on the tail, about 160μ from the tip. By the 6th week the constriction was marked, and the whole tail appeared to be withering away. This was clearly the beginning of moult 1. Underneath the skin of the first moult another smooth cuticle was observed extending well beyond the extremity of the tail. This appeared to us to be the beginning of moult 2. If so, moult 2 takes place shortly after moult 1.

The anus was situated about 75μ anterior to the constriction. Apart from this shedding of the tail, no other details were noted. Manson (1895) showed that the development of the larvae in cyclops in England took at least 70 days before reaching an infective stage. In the tropics it is much more rapid.

As one or more cyclops were killed every day in order to observe any changes in the larva, our stock of infected cyclops became exhausted, and we were therefore not able to observe the entire development of the larvae up to the infective stage.

REFERENCES

CHATTON, E. (1918*a*). Observations et expériences faites à Gabès sur le ver de Guinée : preuve expérimentale de l'infestation des *Cyclops* par voie intestinale. *Arch. Inst. Pasteur Tunis*, **10**, 158.

——— (1918*b*). Observations sur le ver de Guinée : preuve expérimentale de l'infestation des *Cyclops* par voie digestive. *Bull. Soc. Path. Exot.*, **11**, 338.

FAIRLEY, N. H., and LISTON, W. G. (1924). Studies in the transmission of *Dracunculus medinensis* : a negative experiment. *Ind. Jl. Med. Res.*, **12**, 93.

GURNEY, R. (1933). British fresh-water Copepoda. vol. 3. (*Ray Soc.*) Lond.

ISSAJEV, L. (1934*a*). Ueber die Eindringung der *Dracunculus medinensis*-Larven in den Cyclops. *Med. Parasitol., Moscow*, **3**. [In Russian, p. 212 ; German summary, p. 230.]

——— (1934*b*). Experimentelle Dracunculosis beim Hunde. *Ibid.*, **3**. [In Russian, p. 231 ; German summary, p. 237.]

——— (1934*c*). Einfache Methode zum Nachweis der Nematoden-Larven in den Crustacea. *Ibid.*, **3**. [In Russian, p. 238 ; German summary, p. 240.]

LEIPER, R. T. (1906*a*). The influence of acid on guinea worm larvae encysted in cyclops. *Brit. Med. Jl.*, **1**, 19.

——— (1906*b*). Some results of the infection of monkeys with guinea-worm. Brit. Assoc. for the Advancement of Science. York.

——— (1907). The etiology and prophylaxis of dracontiasis. *Brit. Med. Jl.*, **1**, 129.

MANSON, P. (1895). On the guinea-worm. *Ibid.*, **2**, 1350.

MOORTHY, V. N. (1935). The influence of fresh bile on guinea-worm larvae encysted in cyclops : a preliminary report. *Ind. Med. Gaz.*, **70**, 21.

——— (1938). Observations on the development of *Dracunculus medinensis* larvae in cyclops. *Amer. Jl. Hyg.*, **27**, 437.

——— and SWEET, W.C. (1936*a*). A note on the experimental infection of dogs with dracontiasis. *Ind. Med. Gaz.*, **70**, 437.

——— ——— (1936*b*). Guinea-worm infection of cyclops in nature. *Ibid.*, **71**, 568.

——— ——— (1936*c*). A peculiar type of guinea-worm embryo. *Ind. Jl. Med. Res.*, **24**, 531.

——— ——— (1938). Further notes on the experimental infection of dogs with dracontiasis. *Amer. Jl. Hyg.*, **27**, 301.

RAO, S. S. (1936). The effect of gastric juice and of bile on cyclops infected with guinea-worm larvae. *Ind. Jl. Med. Res.*, **24**, 535.

ROUBAUD, E. (1913). Observations sur la biologie du ver de Guinée : infection intestinale des cyclops. *Bull. Soc. Path. Exot.*, **6**, 281.

——— (1920). Nouvelle contribution à l'histoire du ver de Guinée : essai d'infestation des cyclops de la région parisienne ; absence d'évolution, mais conservation de l'embryon chez *C. viridis* : considerations étiologiques. *Ibid.*, **13**, 254.

TURKHUD, D. A. (1913). Dracontiasis. *Proc. 2nd All-India Sanit. Conf., Madras*, 1912, **3**, 118.

THE CEREBROSPINAL FLUID OF MONKEYS (*CERCOPITHECUS* SP.) INFECTED WITH A STRAIN OF *TRYPANOSOMA RHODESIENSE*

BY

J. F. CORSON

(*Medical Officer at Tinde Laboratory, Tanganyika Territory*)

(*Received for publication June 21st*, 1938)

Peruzzi (1928) examined the cerebrospinal fluid of monkeys (*Cercopithecus* sp.) which were infected with *Trypanosoma rhodesiense*, and got the following results, which are taken from his more detailed records.

Monkey	Duration of the infection, in days	Trypanosomes in the cerebrospinal fluid
351	14	Absent
281	26	Present
282	20	Present
313	31	Present
311	34	Absent

He wrote: 'The trypanosomes in the cerebro-spinal fluid were always non-motile, squat, clumped or with stiff flagella, without perceptible undulation, or reduced to a nucleus. They were, then, abnormal forms, or forms in course of destruction.' These results, though few, suggest that living trypanosomes (*T. rhodesiense*) are not usually found in the cerebrospinal fluid of these monkeys until more than a month after the date of infection of the animal. The strains that he used had been recently isolated from man.

During the year from March, 1937, to March, 1938, monkeys of the same species of *Cercopithecus* (or closely allied to it) as those used by Peruzzi in Uganda, were infected in experiments at Tinde Laboratory, in which a strain of *T. rhodesiense* was being maintained in various antelopes by means of cyclical transmission by *Glossina morsitans*. Boxes of laboratory-bred flies were put on an antelope, usually once only, then transferred to clean animals, generally rabbits, and, about the 17th day after the feed on the antelope, the boxes were transferred to monkeys, infection of which indicated that the boxes contained infective flies. As it was at first intended to note the duration of life of the monkeys, observations to find the dates of infection of the cerebrospinal fluid were not made, but the cerebrospinal fluid was examined in some of the monkeys and the trypanosomes and leucocytes were counted. The monkeys were killed with chloroform, and the cerebrospinal fluid was obtained by suboccipital puncture. The duration of the disease was reckoned from the day when

trypanosomes were found in the monkey's blood, as the exact date of infection was unknown, but the incubation period of this strain in these monkeys was nearly always from four to seven days. The results are shown in the following table, in which the infections are arranged in chronological order.

Monkey	Duration of disease	Living trypanosomes	Leucocytes
81	4 months	61	8
83	4 ,,	90	39
86	4 ,,	5	27
85	4 ,,	0	157
93	3 ,,	100	124
42	2 ,,	12	200
89	7 ,,	90	8,000
116	3 ,,	145	165
118	6 ,,	6	106
120	6 ,,	400	1,600
91	5 ,,	200	300
92	5½ ,,	0	600
107	3 ,,	6	156
96	3½ ,,	0	104
98	3 ,,	45	25
61	2½ ,,	0	87
100	2½ ,,	28	40
126	2 ,,	11	49
127	2 ,,	54	56
128	2 ,,	0*	64
129	2 ,,	13	55
124	2 ,,	0*	89
125	2 ,,	3	85
130	2 ,,	5	162
82	1½ ,,	0	51
132	2 ,,	2	43
138	19 days	0*	211
Control			4
Control			5

*Inoculated rat infected.

The differences in the figures for the same duration of the disease might be due to differences between monkeys or to variations in a monkey from time to time.

The resistance of these monkeys varies, but there is no doubt that they are much less resistant than man to this strain of *T. rhodesiense*; it could not be concluded from this that their cerebrospinal fluid would be infected earlier, though that might reasonably be expected. It is interesting, however, to compare these results with some observations that have been made on man; the latter depend somewhat on the accuracy of the histories given by native African patients and their friends.

Keevill (1928) recorded six cases with trypanosomes in the cerebrospinal fluid, in which the duration of the symptoms before treatment was very short, viz., 3, 8, 10, 11, 14 days and one month. All were cured by a short intensive course of germanin or the identical French preparation, Fourneau 309.

Maclean and Fairbairn (1932) divided their 719 cases into three categories, of which the first comprised cases with histories of illness of not more than one month. These were treated with germanin, either alone or followed by tryparsamide. Of 173 patients who received 4 gm. or more of germanin, 37, or 21 per cent., died; and of 208 patients who received 3 gm. or more of germanin, 49, or 23 per cent., died. Of 74 patients in category I on whom lumbar puncture was done, 11 had trypanosomes in the cerebrospinal fluid, 47 had 10 or more leucocytes, and 27 had less than 10 leucocytes in a cubic millimetre of the fluid.

The authors also give the results of treatment of patients of all three categories who had trypanosomes in the cerebrospinal fluid; they found that four doses of 1 gm. of germanin, given within a period of from 21 to 29 days, cured 13 out of 39 cases. They concluded that, when a patient remained untreated for more than a week after the onset of symptoms, the prognosis is no longer certain, though it is still very good after a delay of about one month.

A mortality of 21 per cent. after treatment with 4 gm. or more of germanin, in cases with a history of not more than one month's illness, is impressive.

The importance of the amount of protein in the cerebrospinal fluid, for the nutrition and life of the trypanosomes which enter it, has been referred to by several writers (Mott, 1906; Peruzzi, 1928; Zschucke, 1932; Fairbairn, 1934). Fairbairn concluded from his observations that 'It would appear that in some patients, giving a short history, the trypanosome infection causes an early damage to the central nervous system (as shown by a high percentage of protein in the cerebrospinal fluid), and that such cases are not curable by Bayer 205 alone, and possibly not curable at all.'

From the observations of Keevill and of Maclean and Fairbairn, it appears that the cerebrospinal fluid may contain trypanosomes very soon after a person becomes infected, and that very early diagnosis and treatment are of great importance. This may have some bearing on the question of the safety of the prophylactic use of germanin; if there is a risk that it might make diagnosis more difficult, and so delay it, the disadvantages may be thought to be greater than the advantages.

REFERENCES

Fairbairn, H. (1934). Lange's colloidal gold reaction and the estimation of total proteins in the cerebrospinal fluid of Rhodesian sleeping sickness, and their significance in prognosis. *Trans. Roy. Soc. Trop. Med. & Hyg.*, **27**, 471.

Keevill, A. J. (1928). *Trypanosoma rhodesiense* infection treated with 'Bayer 205' or 'Fourneau 309.' *Ibid.*, **22**, 83.

Maclean, G., and Fairbairn, H. (1932). Treatment of Rhodesian sleeping sickness with Bayer 205 and tryparsamide: observations on 719 cases. *Ann. Trop. Med. & Parasitol.*, **26**, 157.

Peruzzi, M. (1928). Pathologico-anatomical and serological observations on the trypanosomiases. *Final Rep. of the League of Nations Internat. Comm. on Human Trypanosomiasis*, p. 245. C.H. 629. Geneva.

Zschucke, J. (1932). Beitrag zur Kenntnis der Schlafkrankheit in den westafrikanischen Küstengebieten. *Ztschr. f. Hyg. u. Infekt.*, **114**, 464.

STUDIES IN CHEMOTHERAPY*

XX.—THE PREPARATION OF STRAINS OF TRYPANOSOMES RESISTANT TO SYNTHALIN AND UNDECANE DIAMIDINE, AND AN ANALYSIS OF THEIR CHARACTERS

BY

E. M. LOURIE

AND

WARRINGTON YORKE

(*Received for publication July 1st*, 1938)

In previous papers we have discussed in detail the preparation of strains of trypanosomes resistant to various aromatic arsenical and antimonial compounds, to acriflavine, to tartar emetic and to Bayer 205, respectively. We have also considered the characters exhibited by these resistant strains, and their stability when passaged for many years through mice by blood inoculation, and when transmitted cyclically by Glossina.

We have shown that, whilst resistance to the aromatic arsenicals and antimonials and to acriflavine is quickly acquired, resistance to Bayer 205 is developed very slowly, and that resistance to tartar emetic cannot be produced unless the trypanosome is first made resistant to an aromatic compound of arsenic or antimony.

There are three vague references in the literature to the question of synthalin resistance. Jancsó and Jancsó (1935), who were firmly impressed with the hypothesis that the therapeutic action of synthalin on trypanosome infections was dependent upon a hypoglycaemia produced by the drug, considered the possibility of synthalin resistance, but dismissed it as highly improbable, on the grounds that it was very unlikely that it would be possible to wean the parasites from their dependence upon sugar. So convinced were the Jancsós of the correctness of this idea that they considered it possible that synthalin, in contrast to other chemotherapeutic substances, would not lose its activity after repeated administration. Schern and Artagaveytia-Allende (1936) make the bald statement that they have suceeeded in demonstrating that trypanosomes may become synthalin-fast; no experimental details are given. Browning (1938), as the result of experiments on mice infected with *T. congolense*, writes that ' resistance of the infection to synthalin becomes established, although it remains undecided as to whether this occurs owing to alterations in the parasites or the host.'

We ourselves (King, Lourie and Yorke, 1937), in a recent communication, recorded that trypanosomes do not so readily become resistant to undecane

*This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council.

TABLE I

Showing genesis of an undecane diamidine-resistant strain of *T. rhodesiense* in mice

Mouse 980e	Aug., 1937				Sept.														Oct.						
	21	23	26	30	3	6	7 14	15	16	17 20	21	22	23	24	25	26 27	28	29 30	1 4	5	6 11	12	13 14	15
Infection (parasites per field)	Inoc. with normal	1/50	0	0	0	0	0	1/20	1	0	1/30	1	20	15	1/50	0	1/3	0	0	1	0	1	0	3
Drug (mgm.per 20 gm. mouse)	strain	·05	·05	·05	·05	·05			·05			·05 *	·05	·05 *			·1 *			·1		·1		†

* On these occasions the strain was transferred to other mice and its resistance examined; some of these mice were cleared by ·05 mgm. and others not.

† Strain transferred to Mouse 115k.

Mouse 115k	Oct.							Nov.
	15	16 18	19	20	21 27	28	29 30	1
Infection ...	Inoc.	0	1/10	20	0	10	0	Killed
Drug ...	from 980e			·1		·15 *†		

* On this occasion the strain was transferred to other mice and its resistance examined; it was found to be sensitive to ·05 mgm.

† Strain transferred to Mouse 115p.

Mouse 115p	Oct.	Nov.							Dec.															
	28	31	1	2 8	9	10	11	12 16	17	18	19 21	22	23	24	25	26	27 2	3	4	5	6 7	8	9
Infection ...	Inoc.	+	+++	0	1	++	1	0	5	+	0	3	20	2	5	+	0	20	15	2	1/5	10	++
Drug	from 115k		·15			·15				·15 *			·15			·15 *		·15 †					·25 D

* On these occasions the strain was transferred to other mice and found resistant to ·05 mgm. but sensitive to ·1 mgm.

† Strain transferred to Mouse 196b.

TABLE I—*Continued*

Mouse 196b	Dec. 3	6	7	8 9	10	11	12	13	14 16	17	18	19	20 25	26	27 28	29	30	31
Infection ...	Inoc.	15	10	+	1/5	5	+++	++	0	2	20	5	0	20	1/20	1/20	10	D
Drug ...	from 115p	·05					·25			·25	·25			·25		†	·25	

† Strain passaged through Mice 307o and 307p without treatment and then transferred to 307q.

Mouse 307q	Jan., 1938 3	5	7	8	10 11	12
Infection	Inoc.	3	20	1/5	0	D
Drug ...	from 307p	·25	·25 †			

† Strain transferred to Mouse 350s.

Mouse 350s	Jan. 7	10	11	12	13
Infection	Inoc.	1/40	1	20	D
Drug ...	from 307q		·25	·25 †	

† Strain transferred to Mouse 350v.

Mouse 350v	Jan. 12	14	15	16	17
Infection	Inoc.	20	20	+	D
Drug ...	from 350s	·25	·25 †		

† Strain transferred to Mouse 443y.

Mouse 443y	Jan. 15	18 19	20	21	22 23	24	25 27	28	29	30 31	1 6	7	8	9	10	11	12	13	14	15	16	17
Infection ...	Inoc.	1/50	+	++	0	1/3	0	2	20	0	0	1/5	0	1/2	1/20	1/20	0	1	3	+	++	+++
Drug	from 350v		·25	·25		·25		·25	·25			·25		·25	·25	·25			·25	·25	·25	D †

† Strain transferred to Mouse 443p.

Mouse 443p	Feb. 17	18	19	20	21	22
Infection ...	Inoc.	1/20	10	+	++	+++
Drug ...	from 443y	·25	·25		·25 †	D

† Strain transferred to Mouse 573a.

Mouse 573a	Feb. 21	22	23	24
Infection	Inoc. from 443p	1/10	+	+++
Drug ...		·25	·25 †	D

† Strain transferred to Mouse 573b.

Mouse 573b	Feb. 23	24	25	26	27	28	Mar. 1	2	3
Infection	Inoc. from 573a	0	0	0	0	2	5	+	+++
Drug ...	·25	·25	·25	·25	·25	·25	·25	·25 †	D

† Strain transferred to Mouse 573g.

diamidine as they do to the aromatic arsenicals, since repeated administration of subcurative doses of this drug to infected mice had failed to produce any recognizable resistance on the part of the trypanosomes.

As the subject of drug-resistance is of the greatest importance, not only for an understanding of the mechanism of action of drugs, but also from the point of view of therapy, we decided to attack the problem in respect of synthalin and undecane diamidine more closely than we had hitherto attempted.

PRODUCTION OF AN UNDECANE DIAMIDINE-RESISTANT STRAIN IN MICE

Details of the manner in which the resistant strain was produced are set out in Table I. The work commenced on August 23rd, 1937, by subjecting Mouse 980e, which was infected with our normal strain of *T. rhodesiense*, to repeated small doses (0·05 mgm. per 20 gm.) of the drug. It should be noted that the minimum effective dose (M.E.D.*) for the normal strain is 0·025 mgm. per 20 gm. mouse, and that the minimum curative dose (M.C.D.†) is 0·25 mgm. per 20 gm. mouse.

The first suggestion of developing resistance is seen on September 22nd and 23rd, when 0·05 mgm. failed to clear the blood of Mouse 980e. The degree of resistance at this time must, however, have been very slight, as when the strain was transferred to other mice, on September 22nd, 24th and 28th (as indicated by the asterisks in the table), a dose of 0·05 mgm. usually sufficed to clear the blood of the subinoculated mice for a few days. Since, moreover, the blood of mice inoculated from Mouse 115k a month later (October 28th) was cleared by 0·05 mgm. of the drug, the strain had acquired little or no resistance even at this date.

The first definite evidence of resistance is seen in Mouse 115p. On November 18th and 26th, the infection in subinoculated mice resisted 0·05 mgm., but was sensitive to 0·1 mgm. per 20 gm. mouse. From this point the degree of resistance rapidly increased, so that by the middle of December a single maximum well-tolerated dose of 0·25 mgm. per 20 gm. failed to clear the peripheral blood of infected mice (Mouse 196b) ; and by the middle of February, 1938, maximum tolerated doses given on several consecutive days had no effect on the course of the infection (Mice 443y, 443p and 573a).

In order still further to enhance the resistance, if possible, the strain was passaged through a series of 10 mice, in each of which the maximum tolerated dose of 0·25 mgm. was given on the day of inoculation and daily thereafter until the death of the animal from trypanosomiasis. Although this was continued until April, 1938, the results only differed from those shown by Mouse 573b

---

*M.E.D. = minimum dose which suffices to clear the blood of trypanosomes.

†M.C.D. = minimum dose which suffices to produce a permanent cure in at least 80 per cent. of animals.

in that the incubation period was reduced to a point which was only 24 hours longer than in untreated animals.

TABLE II

Showing the time and dosage required to produce an undecane diamidine-resistant strain of *T. rhodesiense* in mice

Date	Length of time after start	Total amount of undecane diamidine given. mgm. per 20 gm. mouse	Degree of resistance of strain to undecane diamidine. M.E.D. mgm. per 20 gm. mouse	Reference to Table I
Aug. 23, 1937 ...	0	0	0·025	
Sept. 22–28 ...	1 month	0·3–0·45	0·05	Mouse 980e
Oct. 28	2 „	0·85	0·05	„ 115k
Nov. 18-26 ...	3 „	1·15–1·45	0·1	„ 115p
Dec. 17–Jan 5, 1938	4 „	1·9–2·65	>0·25	„ 196b and 307q
Feb. 14–22	6 „	5·65–6·9	>repeated doses of 0·25	„ 443y, 443p and 573a

The above results are summarized in Table II. The information supplied in this and the preceding table shows that the preparation of a strain of trypanosomes completely resistant to undecane diamidine is a somewhat long and tedious matter. It was not until after the lapse of 3 months that the first definite sign of resistance was observed, and not until after 4 months had the resistance reached a point when a single maximum tolerated dose failed to clear the blood of mice. Even then the resistance had not reached its maximum, because maximum tolerated doses, repeated on several consecutive days, caused a temporary disappearance of parasites from the blood of infective mice. After about 6 months continued effort a state of complete resistance was reached, repeated maximum tolerated doses having no effect on the infection in mice.

The slowness with which resistance to undecane diamidine developed reminds one of the difficulties encountered in producing Bayer 205-resistance, and is in striking contrast with the ease and speed with which strains of trypanosomes can be made resistant to the aromatic arsenical and antimonial compounds and to acriflavine.

PRODUCTION OF AN UNDECANE DIAMIDINE-RESISTANT STRAIN IN RABBITS

Attention has already been drawn in a previous paper (King, Lourie and Yorke, 1938) to a rabbit (34c) which was peculiar in that it failed to respond to a course of 2·5 mgm. per kilo. of undecane diamidine given at 3- and 4-day intervals for 5 doses, whereas the other 31 rabbits treated by this or somewhat

similar courses quickly responded, all signs of disease disappearing and the blood becoming negative. Details of the various treatments to which this rabbit was exposed, and the results of tests of the resistance of its strain of trypanosomes in subinoculated mice, are shown in Table III.

TABLE III

Showing the development of an undecane diamidine-resistant strain of *T. rhodesiense* in a rabbit

Date	Details of treatment. mgm. per kilo. of rabbit	Total amount of drug given. mgm. per kilo.	Clinical condition after each course of treatment	Resistance tests in subinoculated mice	
				Date of subinoculation of mice	M.E.D. mgm. per 20 gm. mouse
Oct. 11, 1937	Before treatment	0	Pronounced signs	Oct. 11	0·025
Oct. 12–26	2·5 at 3- to 4-day intervals for 5 doses = 12·5 mgm.	12·5	Slight improvement		
Nov. 2–19	2·5 at 3- to 4-day intervals for 6 doses = 15 mgm.	27·5	Worse	Nov. 20	0·05
Nov. 23–Dec. 8	2·5 daily for 16 doses = 40 mgm.	67·5	Still worse	Dec. 9	0·25
Dec. 9–23	5·0 daily for 15 days = 75 mgm.	142·5	Dying	Dec. 24	>0·25

Why this rabbit was exceptional in failing to respond to the first course of treatment is not obvious. It was certainly in very bad condition when treatment commenced and the course of treatment given was not very intense, but other rabbits apparently equally affected responded to a similar course. Moreover, there is no evidence that the trypanosomes infecting this rabbit quickly developed any appreciable degree of resistance to the drug. The test made in mice subinoculated on November 20th, when the rabbit had received two courses of treatment (total amount of drug 27·5 mgm. per kilo.), revealed only very little, if any, resistance, so small a dose as 0·05 mgm. sufficing to clear the blood of the infected mice. A pronounced degree of resistance on the part of the trypanosomes was first noticed in mice subinoculated on December 9th, when the rabbit had received, during a period of 2 months, the colossal amount of 67·5 mgm. of undecane diamidine ; at this point the maximum tolerated dose of 0·25 mgm. per 20 gm. was required to clear the blood of infected mice. When the last test was made on December 24th, by which time the rabbit had received 142·5 mgm.

per kilo. of the drug, the resistance had been so far enhanced that the maximum tolerated dose of 0·25 mgm. per 20 gm. either no longer sufficed to clear the blood of mice or, in exceptional instances, did so for one or two days only.

It is also mentioned in our previous paper (Study XIX) that two other rabbits (76f and 77f), in which the initial courses of treatment were relatively small, eventually developed resistant strains of trypanosomes. These animals, however, differ from that just discussed (Rabbit 34c) in that they responded to the initial course of treatment and subsequently relapsed, whereas the latter failed to respond to treatment from the very beginning.

PRODUCTION OF A SYNTHALIN-RESISTANT STRAIN IN RABBITS

Details of the production of a synthalin-resistant strain of *T. rhodesiense* in a rabbit are shown in Table IV. As was stated in a previous paper (King, Lourie and Yorke, 1938), we have found it impossible to cure rabbits, infected with our strain of *T. rhodesiense*, by means of this drug. Frequently, as is shown in Table IV, a course of the maximum tolerated doses of synthalin results in great improvement, and sometimes in the complete disappearance of the signs of

TABLE IV

Showing the development of a synthalin-resistant strain of *T. rhodesiense* in a rabbit

Date	Details of treatment. mgm. per kilo. of rabbit	Total amount of drug given. mgm. per kilo.	Clinical condition after each course of treatment	Resistance tests in subinoculated mice	
				Date of subinoculation of mice	M.E.D. mgm. per 20 gm. mouse
Oct. 11, 1937	Before treatment	0	Moderate signs of disease		0·05
Oct. 12–26	2·5 at 3- to 4-day intervals for 5 doses = 12·5 mgm.	12·5	Normal, but relapsed in a week		
Nov. 2-16	2·5 at 3- to 4-day intervals for 5 doses = 12·5 mgm.	25·0	Normal, but relapsed in a few days		
Nov. 23–Dec. 7	2·5 at 3- to 4-day intervals for 5 doses = 12·5 mgm.	37·5	Normal, but relapsed in a week		
Dec. 14–Jan. 4, 1938	2·5 at 3- to 4-day intervals for 7 doses = 17·5 mgm.	55·0	Moderate signs of disease	Jan. 5	>0·125
Jan. 6			Died from toxic action of drug		

disease; but a relapse soon occurs. Repetition of the treatment may again result in disappearance of signs of disease, but eventually a state of affairs is reached when either the disease progresses unchecked to a fatal termination, or the animal dies of the toxic effects of the drug.

Unfortunately, in the instance shown in Table IV, the resistance of the trypanosomes to synthalin was not examined until January 5th, at the end of the 4th course of treatment; but as each of the three earlier courses had resulted in a return of the animal to a normal, or practically normal, state there could hardly have been any appreciable increase in drug-resistance on the part of the infection. The 4th course, however, had failed to cause improvement—in fact, the signs of disease had obviously increased. Mice were therefore sub-inoculated at this point and the degree of synthalin-resistance was determined. It was found that 0·125 mgm. per 20 gm. mouse failed to cause the disappearance of parasites from the blood in 4 of 5 mice; in the 5th mouse the parasites disappeared for 3 days; a dose of 0·05 mgm. per 20 gm. had no effect on the infection in any of 5 mice.

These figures show that on January 5th the trypanosomes had acquired a very definite degree of resistance to synthalin. The resistance, however, was not complete, but, unfortunately, it was impossible to continue the experiment with the object of still further enhancing the degree of resistance, because the rabbit died the following day from the toxic effects of the drug.

The results of this work in rabbits are in general agreement with those obtained from mice. In both animals it was found possible to produce a strain of trypanosomes resistant to aliphatic diamidine and diguanidine compounds, but the development of such strains was, in contrast with the production of strains resistant to aromatic compounds of arsenic and antimony and to acriflavine, a rather long and tedious matter.

PRODUCTION OF AN UNDECANE DIAMIDINE-RESISTANT STRAIN BY CONTACT OF TRYPANOSOMES WITH THE DRUG *IN VITRO*

Further evidence of the slowness with which undecane diamidine-resistance develops was obtained in an experiment in which this was produced by subjecting our normal strain of *T. rhodesiense* to contact with the drug *in vitro*. The method adopted was similar to that described in an earlier paper (Yorke, Murgatroyd and Hawking, 1931), in which an account was given of the production of a drug-fast strain by contact *in vitro* of trypanosomes with reduced tryparsamide.

Trypanosomes from a very heavily infected mouse were washed in a medium consisting of equal parts of Ringer-glucose solution and guinea-pig serum, and were then suspended in different concentrations of undecane diamidine in this medium at 37° C. The density of trypanosomes in each drug-containing tube at the beginning was between 20,000 and 30,000 per c.mm. After four hours the trypanosomes were washed three times in medium in order to free them from all traces of drug, and were then inoculated into mice. It was found

that the parasites had survived the *in vitro* treatment only when the concentration of undecane diamidine was not greater than 1 : 1,000,000. Mice infected with trypanosomes which had survived exposure to this concentration were then treated with undecane diamidine, in order to determine whether any resistance had developed.

This whole procedure was then repeated with trypanosomes from a mouse infected with the parasites which had survived the previous exposure to 1 : 1,000,000 undecane diamidine. The sequence of *in vitro* contact of trypanosomes with undecane diamidine, followed by the testing of their resistance *in vivo*, was repeated 9 times, the trypanosomes for incubation *in vitro* on each occasion being descended from survivors in the tube containing the highest concentration of drug which had failed to kill all the organisms at the preceding exposure.

The result of the whole experiment, which occupied about 100 days, was as follows. The first indication of the development of undecane diamidine-resistance appeared after the 4th *in vitro* exposure to the drug, when the minimum effective dose was found to have been increased from 0·025 to 0·075 mgm. per 20 gm. mouse; it was only at the 6th exposure that the trypanosomes were found to survive contact with a higher concentration of drug (1 : 250,000) than they did at the start of the experiment (1 : 1,000,000). After as many as 10 exposures of four hours each, this slight degree of resistance had not been obviously increased. The maximum concentration which the trypanosomes were able to resist *in vitro* was still only 4 times as great (1 : 250,000) as at the start, and the minimum effective dose in infected mice was still no higher than 0·075 mgm.; a dose of 0·25 mgm. (the maximum tolerated dose) per 20 gm. mouse, which suffices to produce a large proportion of cures in mice infected with the normal strain, did not bring about any cures in infections with the resistant parasite.

In striking contrast to the above is the speed and completeness with which drug-resistance was produced in an experiment exactly similar to the foregoing except that the aromatic arsenical, halarsol, was used instead of undecane diamidine. After only 4 *in vitro* exposures of four hours each, at 37° C. (the experiment covering a period of about 40 days), the trypanosomes had acquired the power of surviving a concentration of the drug as much as 64 times as great as they did at the beginning of the experiment—1 : 1,000,000 compared with 1 : 64,000,000*; and infections of trypanosomes at this stage were already completely resistant to the highest tolerated dose of halarsol, 0·6 mgm., although the minimum curative dose of this drug against infections with the normal strain

*It may be noted, in passing, that the lethal concentrations of halarsol *in vitro*, under the conditions of this experiment, are considerably lower than those of undecane diamidine. Using smaller numbers of trypanosomes, and with an observation period of 24 hours, however, the trypanocidal titre of both drugs against normal *T. rhodesiense* was found to be the same—1 : 256,000,000 (see King, Lourie and Yorke, 1937).

is 0·1 mgm., and the minimum effective dose is as low as 0·02 mgm. per 20 gm. mouse.

The fact that trypanosomes do not so readily acquire resistance to undecane diamidine and synthalin as to the aromatic arsenicals has a practical significance, which is well illustrated in a previous paper of this series (King, Lourie and Yorke, 1938). In this paper we give details of a considerable series of rabbits treated by various courses of undecane diamidine. Those animals which relapsed after the first course of treatment were subjected to an exactly similar second course of treatment; the results were remarkable in that a higher proportion of animals were cured by the second course than by the first course. This is the reverse of what we should expect with the aromatic arsenicals, and is clear indication that the first course of treatment had failed to enhance the resistance of the infection to undecane diamidine.

COMPARISON OF UNDECANE DIAMIDINE- AND SYNTHALIN-RESISTANT STRAINS WITH BAYER 205- AND TRYPARSAMIDE-RESISTANT STRAINS OF THE SAME TRYPANOSOME

Having succeeded in preparing strains of *T. rhodesiense* resistant to undecane diamidine and to synthalin respectively, it was decided to examine their reactions to various drugs and to contrast their behaviour with that of other resistant strains of the same parasite. The strains used in these experiments were all prepared from our old laboratory strain of *T. rhodesiense*:

1. *Normal parent strain.* This strain was isolated from man in 1923, and has since been passaged through mice (Yorke, Adams and Murgatroyd, 1930).
2. *Synthalin-resistant strain* developed in the rabbit.
3. *Undecane diamidine-resistant strain* developed in the mouse.
4. *Bayer* 205*-resistant strain* developed in the mouse by Hawking in 1937, and recently exposed by us to repeated doses of Bayer 205 until the resistance had been so enhanced that the colossal dose of 10 mgm. per 20 gm. failed to cause the trypanosomes to disappear from the blood of infected mice.
5. *Tryparsamide-resistant strain* developed in the mouse in 1931 and since maintained by passage through mice (Murgatroyd and Yorke, 1937).

The response of each of the above-mentioned strains in mice was examined in respect of the following compounds—synthalin, n. undecane-1 : 11-diamidine, pp′-diguanidino-diphenyl-methane, naphthalene-2 : 7-diamidine, Bayer 205 and halarsol. The results are summarized in Table V.

The sensitiveness of the resistant strains to the various compounds *in vitro* was also examined, and the results of these experiments are summarized in Table VI.

From the data presented in these two tables the following conclusions can be drawn:

1. The synthalin- and undecane diamidine-resistant strains are indistinguishable from one another in that they are both resistant to each of these drugs and to pp′-diguanidino-diphenyl-methane and naphthalene-2:7-diamidine.

TABLE V

Showing the response of undecane diamidine-, Bayer 205-, synthalin- and tryparsamide-resistant strains in mice to various drugs

Strain	Synthalin		Undecane diamidine		Diguanidino-diphenyl-methane		Naphthalene-2 : 7-diamidine		Bayer 205		Halarsol	
	M.T.D. = 0·125		M.T.D. = 0·25		M.T.D. = 1·0		M.T.D. = 1·0		M.T.D. = 5·0		M.T.D. = 0·6	
	M.E.D.	M.C.D.	M.E.D.	M.C.D.	M.E.D.	M.C.D.	M.E.D.	M.C.D.	M.E.D.	M.C.D.	M.E.D.	M.C.D.
Normal	0·05	0·125+	0·025	0·25	0·1	0·5-1·0	0·025	0·15-0·25	0·025	0·1	0·025	0·1
Synthalin-resistant ...	0·125+	—	0·1-0·25	—	0·5	—	0·5	—	0·025	0·1	0·025	0·1
Undecane diamidine-resistant	0·125+	—	0·25+	—	1·0+	—	1·0	—	0·025	0·1	0·025	0·1
Bayer-resistant ...			0·025	0·15					5·0 +	—	0·025	0·1
Tryparsamide-resistant			0·025	0·15					0·025	0·1	0·6+	—

M.T.D. = maximum tolerated dose. M.E.D. = minimum effective dose. M.C.D. = minimum curative dose.

TABLE VI

Showing the concentration of various drugs necessary to destroy *in vitro*, in 24 hours at 37° C., *T. rhodesiense* of the normal and of certain resistant strains respectively

Strain of trypanosomes	Synthalin	Undecane diamidine	Diguanidino-diphenyl-methane	Diamidino-diphenyl-methane	Naphthalene-2 : 7-diamidine	Halarsol
Normal ...	1 : 64,000,000/256,000,000	1 : 64,000,000/256,000,000	1 : 16,000,000	1 : 4,000,000/16,000,000	1 : 4,000,000/16,000,000	1 : 64,000,000/256,000,000
Undecane diamidine-resistant	1 : 16,000,000	1 : 4,000,000/16,000,000	1 : 1,000,000/4,000,000	1 : 1,000,000	1 : 256,000/1,000,000	1 : 64,000,000/256,000,000
Synthalin-resistant	1 : 16,000,000	1 : 4,000,000/16,000,000	1 : 4,000,000	1 : 256,000/1,000,000	1 : 256,000/1,000,000	1 : 64,000,000/256,000,000
Tryparsamide-resistant	1 : 64,000,000/256,000,000	1 : 64,000,000/256,000,000	1 : 16,000,000	1 : 4,000,000/16,000,000	1 : 4,000,000/16,000,000	1 : 4,000,000

2. Both these strains are, however, as sensitive to Bayer 205 and to halarsol as is the normal parent strain ; and conversely

3. The Bayer 205 and halarsol strains are just as sensitive to undecane diamidine as is the normal parent strain.

In our work on the development of resistance in trypanosomes to various therapeutically active substances we have so far encountered four different types of resistance.

1. Resistance to the aromatic arsenical and antimonial compounds and to acriflavine. This type of resistance can easily be produced by any of the commonly used aromatic compounds of arsenic or antimony and by acriflavine. Trypanosomes possessing this type of resistance are sensitive to tartar emetic, to Bayer 205, and to the amidine and guanidine compounds.

2. Resistance to tartar emetic. In our experience this cannot be produced directly from the parent strain. It is, however, very rapidly acquired by a strain previously made resistant to the aromatic compounds of arsenic or antimony or to acriflavine.

3. Resistance to Bayer 205. Trypanosomes possessing this type of resistance are sensitive to the aromatic compounds of arsenic and antimony, to acriflavine, to tartar emetic, and to the amidine and guanidine compounds.

4. Resistance to the amidine and guanidine compounds. Trypanosomes possessing this type of resistance are sensitive to the aromatic compounds of arsenic and antimony, and to Bayer 205.

SUMMARY

1. The preparation of strains of *T. rhodesiense* resistant to synthalin and to n. undecane-1 : 11-diamidine respectively is described in detail.

2. The development of synthalin- and undecane diamidine-resistance is a slow process as compared with the development of resistance to the aromatic compounds of arsenic or antimony or to acriflavine.

3. The characters of these two strains were examined and compared with those of strains of the same trypanosome which had been made resistant to tryparsamide and to Bayer 205 respectively.

4. It was found that the synthalin- and undecane diamidine-resistant strains are indistinguishable from one another in that they are both resistant to each of these drugs and to pp′-diguanidino-diphenyl-methane and naphthalene-2 : 7-diamidine. Both these strains are, however, as sensitive to Bayer 205 and to halarsol as is the normal parent strain ; and, conversely, the Bayer 205- and tryparsamide-resistant strains are just as sensitive to undecane diamidine as is the normal parent strain.

5. The characters of the four different types of resistance to therapeutic substances which we have developed in *T. rhodesiense* are discussed. These types of resistance are :

(*a*) Resistance to the aromatic compounds of arsenic and antimony and to acriflavine.

(*b*) Superadded tartar emetic-resistance.

(*c*) Resistance to Bayer 205.

(*d*) Resistance to the amidine and guanidine compounds.

Each of these types of resistance is specific for its own group of chemical compounds.

REFERENCES

BROWNING, P. (1938). The chemotherapeutic action of synthalin in experimental infections with *T. brucei* and *T. congolense*. *Jl. Path. & Bact.*, **46**, 323.

JANCSÓ, N. VON, and JANCSÓ, H. VON (1935). Chemotherapeutische Wirkung und Kohlehydratstoffwechsel: die Heilwirkung von Guanidinderivaten auf die Trypanosomeninfektion. *Ztschr. f. Immunitätsforsch.*, **86**, 1.

KING, H., LOURIE, E. M., and YORKE, W. (1937). New trypanocidal substances. *Lancet*, **233**, 1360.

——— ——— ——— (1938). Studies in chemotherapy. XIX: Further report on new trypanocidal substances. *Ann. Trop. Med. & Parasitol.*, **32**, 177.

MURGATROYD, F., and YORKE, W. (1937). Studies in chemotherapy. XIV: The stability of drug-resistance in trypanosomes. *Ibid.*, **31**, 165.

SCHERN, K., and ARTAGAVEYTIA-ALLENDE, R. (1936). Zur glykopriven Therapie und Prophylaxe mit sowohl toxisch als auch atoxisch wirkenden Substanzen bei der experimentellen Trypanosomen- und Treponemen-Infektion. *Ztschr. f. Immunitätsforsch.*, **89**, 21.

YORKE, W., ADAMS, A. R. D., and MURGATROYD, F. (1930). Studies in chemotherapy. II: The action *in vitro* of normal human serum on the pathogenic trypanosomes, and its significance. *Ann. Trop. Med. & Parasitol.*, **24**, 115.

——— MURGATROYD, F., and HAWKING, F. (1931). Studies in chemotherapy. VI: The production of resistant strains by exposure of trypanosomes to reduced tryparsamide *in vitro*. *Ibid.*, **25**, 521.

Printed in Great Britain by H. R. GRUBB, LTD., Croydon.

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

12th October, 1938 Vol. 32. No. 3

Annals of Tropical Medicine and Parasitology

ANNALS
Edited by
Prof. W. Yorke
Prof. D. B. Blacklock
Prof. R. M. Gordon
Dr. F. Schwetz
Prof. T. H. Davey

PUBLISHED BY
THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Administered by

hæmolytic strep[illegible] infections

TRADE MARK

'TABLOID'

SULPHONAMIDE

0·5 grams

(*p*-aminobenzenesulphonamide [illegible])

Successfully employed in Hæmolytic Streptococcal Infections such as :—

Streptococcal Septicæmia, [illegible] Puerperal Septicæmia, Scarlet Fever with Complications, Erysipelas, Tonsillitis.

Also in Meningococcal, Gonococcal and in Urinary Tract Infections such as *B. coli* and *Proteus ammoniæ*.

Trial is also justified in :—

Staphylococcal Infections, Typhoid and Para-typhoid Fevers.

Literature to Medical Men on request.

Bottles of 25 products, at 2/- per bottle. Bottles of [illegible] at higher [illegible]

London Prices [illegible]

BURROUGHS WELLCOME & CO., LONDON

D 610

A BARTONELLA OF THE GUINEA-PIG, *BARTONELLA TYZZERI* SP. NOV.*

BY

DAVID WEINMAN, M.D.

AND

HENRY PINKERTON, M.D.

(*From the Department of Comparative Pathology and the Department of Pathology, Harvard Medical School, Boston, Massachusetts*)

(*Received for publication July 11th*, 1938)

INTRODUCTION AND REVIEW OF LITERATURE

Bartonella, a genus created in 1915 (see Strong *et al.*) for the aetiological agent of Oroya fever, is represented by a group of minute bacterium-like micro-organisms which infect the erythrocytes of man and a number of lower animals and which possess certain common biological characters. The most carefully studied of the bartonellae are the human bartonella (*B. bacilliformis* ; see Strong *et al.*, 1915), the rat bartonella (*B. muris* ; see Mayer, 1921) and the dog bartonella (*B. canis* ; see Kikuth, 1929). A critical review of the subject has recently been published by one of us (Weinman, 1935).

B. bacilliformis has been transmitted to monkeys, and there is some evidence that *B. muris* may infect the rabbit (Ford and Eliot, 1928), but in general the different species of bartonellae show a high degree of specificity with respect to their mammalian hosts, and it is largely on this basis that they can be distinguished from one another.

Guinea-pigs have been extensively studied for bartonella infection by workers in various parts of the world. Review of the literature on this question reveals a record of incomplete observations and unconvincing conclusions. Special difficulties beset purely morphological studies of bartonelliform bodies in the guinea-pig, particularly when these bodies are present in small numbers and are atypical in structure. Normal guinea-pigs present in their erythrocytes the structures described by Wenyon and Low (1914) and considered by them to be non-parasitic in nature, probably representing nuclear remnants. Certain of these structures may resemble some forms of bartonellae ; therefore there is a possibility that the two might be confused by inexperienced workers.

Mayer and Zeiss (1920) infected cavies with *Trypanosoma rhodesiense* and then treated them with Bayer 205. During treatment structures appeared in the blood which had the appearance of bartonellae, but which the authors explained as being the result either of the action of the drug or of the disintegrating trypanosomes. Since identical treatment of rats latently infected with *Bartonella muris* provokes the appearance of this parasite in the blood, it is

* A continuation of the investigations of the 1937 Harvard Expedition to Peru.

possible that Mayer and Zeiss were in reality dealing with guinea-pigs carrying a spontaneous bartonella infection.

Campanacci (1929) inoculated lead-poisoned guinea-pigs with *Staphylococcus aureus*. Subsequently 'azurophile inclusions,' occurring usually in hypochromic red cells, were observed. No illustration of these structures was given. The author stated that he was not certain whether or not they were parasites, and against their parasitic nature he urged the fact that the same procedure always produced other endoglobular products in addition to these structures. Although no evidence was presented that these structures were living organisms, the name *Bartonella caviae* was proposed for them. Since the nature of these objects is unknown, *B. caviae* is, at present, an unacceptable biological species.

Rybinsky (1929) did not find bartonellae in splenectomized guinea-pigs. Another group of guinea-pigs (not splenectomized) received injections of trypan blue, and some were also infected with *Trypanosoma brucei*. Subsequently there appeared in the red blood cells of some of these inoculated guinea-pigs structures which stained dark violet with Giemsa's solution, were round or oval in shape, and varied in size from 1μ to 2μ. The author named these structures *Bartonella ukrainica*. There was no attempt to transmit them from one animal to another, nor were they cultured. From the description furnished (no illustration is given) it does not appear that the bodies resemble bartonellae. It has not been shown that they are parasites, much less that they are bartonellae. *B. ukrainica*, therefore, should not at present be accepted as a valid species, until it can be determined precisely what the structures seen by Rybinsky represent.

Klein, Lopatizki and Soliterman (1930) utilized splenectomized guinea-pigs. In these animals they observed structures in the red cells and more rarely in the plasma. These bodies were almost exclusively coccus-shaped, with some rare rod forms. No more than two of these structures were seen in any one red cell. Inoculation of non-splenectomized guinea-pigs with typhoid cultures and with blood containing these bodies was followed by their reappearance in the inoculated animals, sometimes accompanied by a slight anaemia. The authors considered these bodies to represent a species of bartonella, which, however, they did not name.

A critical review by Schwetz of the work of the last-cited authors appeared in 1934. From careful experiments of his own he concluded that red-staining inclusions may occur in the erythrocytes of normal guinea-pigs, usually appearing as dots or irregular forms and on which splenectomy has no notable influence; that these inclusions are probably nuclear remnants and seem to be identical with those seen by Wenyon and Low; and that, awaiting proof of the contrary, that is to say, crucial experiments, it has not yet been shown that guinea-pigs are parasitized by a bartonella of their own. This judgement of Schwetz seems to be fully justified and to apply equally well to the work of all his predecessors which we have discussed here. The essential features of the bartonellae are

infection of erythrocytes, morphological similarity to the type species (*B. bacilliformis*), and inoculability to uninfected animals of the same species. In order to demonstrate that structures seen in erythrocytes are bartonellae, it should be shown that they satisfy all the above criteria and that they are morphologically distinguishable from non-living structures with which they might be confused. Furthermore, the appearance or increase of organisms in the blood-stream after splenectomy, although not demonstrated for *Bartonella bacilliformis*, is so constant with animal bartonellae that one would hesitate to place an organism in this group in the absence of this phenomenon.

A bartonella infection of the guinea-pig satisfying all the above criteria, and cultivable on cell-free media, was found by the present authors in guinea-pigs obtained in Peru, and has been briefly reported (Weinman and Pinkerton, 1937). Since the guinea-pigs in which this bartonella infection was discovered were native to the Andean region—the only part of the world in which human bartonella infection is known to be contracted—and in view of the fact that no unquestionable bartonella infection had previously been reported in the guinea-pigs, notwithstanding the general utilization of this animal by laboratory workers throughout the world, the possibility was for a time entertained that the bartonella found in Peruvian guinea-pigs might be identical with *B. bacilliformis*. The objects of this report are to describe the guinea-pig bartonella in detail, to show that it is distinct from *B. bacilliformis*, *B. muris* and *B. canis*, and to classify it on the basis of its biological reactions. Since the descriptive name *B. caviae* has been applied to other unrelated structures in the erythrocytes of the guinea-pigs (Campanacci, 1929), the name *B. tyzzeri** has been chosen for it.

THE HOST AND THE PARASITE

B. tyzzeri was first noted in Peruvian guinea-pigs (*Cavia porcellus*) in Lima, Peru. These animals were all bred in Peru, although their precise origin could not be determined. Subsequently, cavies obtained from farms situated in the regions where human bartonellosis occurs were also found to be infected.

Like other recognized species of bartonella, the organism shows a high degree of host specificity. Splenectomized bartonella-free guinea-pigs were infected by blood or cultures injected subcutaneously or intraperitoneally. The incubation period, from inoculation to the appearance of the organisms in the blood, was then from 12 to 15 days, about double that required when latently infected guinea-pigs were splenectomized; in all other respects the course of the infection was identical. Five splenectomized *Bartonella muris*-free rats did not develop any visible infection when inoculated with infected guinea-pig blood. *Macacus rhesus* monkeys were inoculated, one with infected blood, the other with infected tissue, and later reinoculated with luxuriant 10-day cultures. Neither of these last-named animals has shown visible infection or given

* In recognition of the important contributions of Dr. Ernest Edward Tyzzer.

positive blood cultures throughout a period of several months after inoculation.

The organism, like other species of bartonella of the lower animals, has been observed in the blood of latently infected animals only after splenectomy. Thus, in the blood of 15 non-splenectomized Peruvian guinea-pigs, this parasite was not observed, whereas it was found in the blood-films of 4 (or about 17 per cent.) of 23 animals after splenectomy. Infected red blood cells were first observed to appear from 5 to 9 days after splenectomy, the parasites remained visible in blood-films for a period varying between a few days and a month, and then they could not be seen, although the blood remained infectious for at least two months. Infected red cells were not numerous, and only about 0·1 per cent. of the cells were infected in the more intense infections. Each infected red cell carried from 1 to 15 organisms, but otherwise appeared normal in shape, haemoglobin-content and in staining reaction (Plate III, fig. 1).

No mortality has resulted from this infection, which is extremely well tolerated. Jolly bodies and polychromatophil cells were found in the blood, but no more frequently than is customary in splenectomized non-infected animals. No blood cells indicative of significant anaemia were regularly encountered. Erythrophagocytosis of infected cells was rarely observed, and haemoglobinuria did not occur. No verrucous lesions resulted from subcutaneous or intracutaneous inoculations of guinea-pigs, and scrotal swelling was not observed after intraperitoneal or subcutaneous inoculation. The organism was not encountered within vascular endothelial cells of spleen, liver, lung or kidney examined in Giemsa-stained films, nor in sections of these organs after Regaud fixation and Giemsa staining.

In the blood the organism stained intensely red-violet with Giemsa or May-Grünwald Giemsa solutions. It then appeared as single or composite rods, varying from 1·4μ to 4·0μ in length, and about 0·25μ in width. The surface of the rods was most often smoothly regular, but occasionally showed granular swellings. The poles were frequently enlarged. Short rods averaging 0·8μ × 0·2–0·3μ also occurred. Round forms with a diameter of 0·2–0·3μ were found inconstantly; in some animals they were frequent and then usually occurred in pairs, in other animals they have not been observed. The predominating size or shape varied slightly in the same animal from day to day, but without any determined sequence. The distribution of the organism in the red cells was irregular; no clumps or rafts have been seen. No organisms have been encountered in leucocytes except for those infecting phagocyted red cells. No evidence of a complex life-cycle has been observed, and no differentiation between nucleus and cytoplasm was evident with the stains employed. Reproduction in the blood presumably takes place through simple division.

CULTIVATION EXPERIMENTS

Initial cultures of *B. tyzzeri* have been obtained on Noguchi's semi-solid medium (Noguchi and Battistini, 1926) in four cases, while in seven other cases,

with organisms in the erythrocytes, negative results were obtained. Tubes of this medium inoculated with citrated blood and incubated at 28°C. commonly developed colonies in two to three weeks. The latter appeared as numerous white isolated spheres, not growing much larger than 1 mm. in diameter, which developed in a zone of varying width, the upper border of which was about 8 mm. below the surface of the medium.

The organisms in such cultures occurred in microscopic clumps, which usually measured about 10–20μ from edge to edge. Individual organisms were most clearly seen at the periphery of these aggregations. Giemsa's solution stained them ' azur ' or more rarely a redder violet ; they were decolorized with Gram's technique. The clumps were composed of rods and granules, so that the appearance was entirely comparable to that of *B. bacilliformis* in culture. Interspersed among these two forms of the organism, larger round structures or discs occurred inconstantly (Plate III, fig. 2). These discs have not been observed in all cultures, nor in all clumps of the cultures where they did occur. They varied from 0·5μ to 2·0μ in diameter, and discs of varying size were found in the same clump. The discs, like the individual organisms, stained a reddish or a bluish violet, and both varieties were usually found in a single clump which contained the discs. At times, these discs were composed of smaller particles which were rod-like or granular (Plate III, fig. 3, *b*). The significance of these discs is not altogether certain. Similar structures are seen in cultures of *B. bacilliformis*. When cultured in association with living tissues, the discs were often seen within the cytoplasm of cells heavily infected with rod forms.

For purposes of comparison with *B. bacilliformis*, the behaviour of *B. tyzzeri* in the presence of surviving guinea-pig mesenchymal cells was determined. For this purpose, the agar-slant modification of the Nigg-Landsteiner-Maitland method recently devised by Zinsser, Wei and Fitzpatrick (1937) was employed. Minced tunica vaginalis from a normal guinea-pig was infected by soaking in 2-3 drops of blood from an infected guinea-pig, in which about 0·1 per cent. of the red cells were lightly infected. This tunica tissue was then spread on the surface of the medium. After 6–8 days, fragments of tunica tissue were smeared and stained with Giemsa's solution. Many cells were enormously distended with clumps of bartonellae, which were sometimes quite discrete (Plate III, fig. 3, *a*) and at other times fused into nearly homogeneous plaques. These clumps ranged from 2μ to 8μ in diameter, and the picture obtained was an exact duplication of that obtained with *B. bacilliformis* (Pinkerton and Weinman, 1937).

As in the case of *B. bacilliformis*, multiplication occurred extracellularly as well as intracellularly, producing, by about the 15th day, barely visible pin-point colonies on the surface of the medium. This experiment emphasizes the biological similarity between *B. tyzzeri* and *B. bacilliformis* and furnishes additional reason for placing these two organisms in the same genus. It is to be noted that this pattern of intracellular clustering occurs both *in vivo* and

in vitro with *B. bacilliformis*, but is brought out only by *in vitro* experiments in the case of *B. tyzzeri*.

Cultures of *B. tyzzeri* are infective for guinea-pigs. On semi-solid medium, they remain so for at least eight weeks, and will retain their infectiousness after two weeks' refrigeration at about 10°C. The incubation period after the inoculation of cultures on both types of medium is the same as for infective blood.

SYSTEMATIC POSITION OF THE PARASITE

The assignment of the parasite discussed in this paper to a definite systematic position was made with full realization of the fact that such generic names as *Bartonella* and *Rickettsia* are quite arbitrarily defined. Morphologically and tinctorially, the bartonellae greatly resemble the rickettsiae, and the similarities between these two groups of organisms have been stressed by various workers. Bartonella infections are presumably arthropod-transmitted, and if intracellular multiplication in arthropod tissues should be demonstrated the analogy would become even closer. In the absence of more exact criteria, however, these minute organisms must be classified largely on the basis of their biological reactions. The bartonellae differ from the pathogenic rickettsiae in various ways—notably in their ability to infect erythrocytes, and to grow extracellularly as well as intracellularly. The pathogenic effects of the two types of organisms are also quite different. For these reasons, both genera appear to be valid at the present time.

From the appearance of the organism described here, and from the fact that it is a parasite of erythrocytes, only two genera may be considered for it, that is, *Bartonella* and *Grahamella*. Differential criteria between the two have been summarized by Bruynoghe and Vassiliadis (1929). The organism under discussion resembles other bartonellae and differs from the grahamellae in that it increases markedly after splenectomy and is inoculable to animals of the same species. Furthermore, its polymorphism, although not marked, is somewhat greater than that of the grahamellae ; the number of organisms found in infected cells is more variable than in the grahamella infections, and it has been obtained in culture, while to date none of the grahamellae have been cultivated. Finally, in appearance it closely resembles *B. bacilliformis*, the type species of the genus as originally described (Strong *et al.*, 1915).

The recognition of the guinea-pig bartonella as a distinct species is made with the knowledge that three well-studied species of bartonella, *B. bacilliformis*, *B. muris* and *B. canis*, have been reported from Peru. The bartonella of the guinea-pig is biologically distinct from all three on the basis of the following features. Cultures of *B. bacilliformis* injected intraperitoneally into six bartonella-free splenectomized guinea-pigs did not result in the appearance of parasites in the blood. Since the guinea-pig may be regularly infected with the guinea-pig bartonella and has never been infected with *B. bacilliformis*, this difference in hosts would suffice to separate the two organisms. Moreover, *M. rhesus* monkeys

have not been successfully inoculated with *B. tyzzeri* (see above), whereas they are easily infected with *B. bacilliformis*. Splenectomized guinea-pigs have not been successfully inoculated with *B. canis* (Kikuth, 1929). Perhaps even weightier evidence against the identity of *B. canis* and *B. tyzzeri* is derived from their morphological dissimilarity, which is so great that the two species might be considered morphologically at opposite extremes of the bartonella series.

Finally, *B. tyzzeri* can also be distinguished from *B. muris* by cross-inoculation tests. Splenectomized *Bartonella muris*-free rats have not been successfully inoculated with *B. tyzzeri* (see above); conversely, according to Noguchi (1928), Amako (1930), Kirschner and Timmerman (1930), and Cossali (1932), the splenectomized guinea-pig cannot be infected with *B. muris*.

The diagnostic features of *B. tyzzeri* may be recapitulated as follows: infection of the erythrocytes of the guinea-pig (*Cavia porcellus*); morphological similarity to *B. bacilliformis*, comprising long and short rods and coccoid forms; growth like *B. bacilliformis* in compact clusters in guinea-pig mesenchymal cells *in vitro*; development within mesenchymal cells in infected guinea-pigs not observed; production of a latent infection in the guinea-pig which may be rendered patent by splenectomy; transmissibility from guinea-pig to guinea-pig but not to *Macacus rhesus* monkeys or albino rats; multiplication *in vitro* in suitable cell-free media.

DISCUSSION

Although infection with *B. tyzzeri* is sub-clinical in its manifestations, the benignity of the infection may be due largely to the small numbers of parasites found in the blood at any time. *B. bacilliformis* and *B. muris* often infect nearly every erythrocyte in their specific hosts, and at such times the anaemia is severe and the resultant mortality high. In the case of *B. muris*, the available evidence indicated that the parasite destroys the red cells by direct action upon them (Weinman, 1938). If *B. tyzzeri* does have similar action, its effect would be insignificant, if even detectable, since not more than one cell in a thousand is parasitized. If all the infected cells were destroyed daily this blood loss would still be far less than that lost normally every day.*

Why so few cells are infected is not known. It may be due to the low ' aggressiveness ' or ' virulence ' of the organism. However, the fact that the spleen acts in the animal bartonelloses by limiting the number of parasites in the blood suggests another possibility, namely, that in the guinea-pig, after splenectomy, some other tissue or organ acts as a partial physiological equivalent of the spleen and thus maintains the number of infected cells at a low level.

The geographical distribution of *Bartonella tyzzeri* does not seem to be as

* By analogy with the findings in human beings, where the normal daily blood loss is calculated at from 1·1 to 7·0 per cent. of the whole blood.

wide as that of the bartonellae of other common laboratory animals. No bartonellae were found in splenectomized guinea-pigs by Noguchi in New York (1928), by Schin in Korea (1930), or by Schwetz in the Belgian Congo (1934). The present authors have splenectomized 18 guinea-pigs obtained in Boston. No bartonella infections have been found among them.

The possibility of spontaneous infection in guinea-pigs with this organism must be borne in mind in working with morphologically similar organisms, such as *Rickettsia prowazeki*. The latter organism, in tissue or Maitland cultures, is distinguishable in heavily infected cells by the absence of clustering, and could also be identified by its pathogenic effect and by its failure to grow in cell-free media.

SUMMARY

1. Guinea-pigs of Peru carry a spontaneous latent bartonella infection.

2. The morphological and biological criteria on the basis of which this guinea-pig bartonella is identified and distinguished from *B. bacilliformis*, *B. muris* and *B. canis* are given in detail.

3. The name *Bartonella tyzzeri* is proposed for this organism.

ACKNOWLEDGEMENTS.—It is a pleasure to acknowledge the aid received from Dr. Glover Allen for the specific identification of the Peruvian guinea-pigs, and from Dr. Serge Elisséeff, who rendered the Russian literature available.

REFERENCES

AMAKO, T. H. (1930). *Bartonella muris* in Japan. *Zentralbl. f. Bakt., Orig.*, **116**, 273.

BRUYNOGHE, R., and VASSILIADIS, P. C. (1929). Différences entre *Bartonella muris ratti* et *Grahamella*. *C. R. Soc. Biol.*, **101**, 150.

CAMPANACCI (1929). *Ateneo Parmense*, **1**, 99.

COSSALI, C. (1932). *Arch. Ist. Biochim. Ital.*, **4**, 297.

FORD, W. W., and ELIOT, C. P. (1928). The transfer of rat anemia to normal animals. *Jl. Exper. Med.*, **48**, 475.

KIKUTH, W. (1929). Ein neuer Anämieerreger. *Zentralbl. f. Bakt., Orig.*, **113**, 1.

KIRSCHNER, L., and TIMMERMAN, W. A. (1930). Untersuchungen über Bartonella-Infektionen der Ratten in Niederländsch-Indien. *Ibid.*, **115**, 437.

KLEIN, B., LOPATIZKI, R., and SOLITERMAN, P. (1930). Beiträge über den Erreger der experimentellen perniziösen Anämie. *Arch. f. Schiffs- u. Trop.-Hyg.*, **34**, 274.

MAYER, M. (1921). Über einige bakterienähnliche Parasiten der Erythrozyten bei Menschen und Tieren. *Ibid.*, **25**, 150.

——— and ZEISS, H. (1920). Versuche mit einem neuen Trypanosomenheilmittel (' Bayer 205 ') bei menschen- und tierpathogenen Trypanosomen. *Ibid.*, **24**, 257.

NOGUCHI, H. (1928). Etiology of Oroya fever. XI : Comparison of *Bartonella bacilliformis* and *Bartonella muris* ; cultivation of *Bacterium murium*, n.sp. *Jl. Exper. Med.*, **47**, 235.

——— and BATTISTINI, T. S. (1926). Etiology of Oroya fever. I : Cultivation of *Bartonella bacilliformis*. *Ibid.*, **43**, 851.

PINKERTON, H., and WEINMAN, D. (1937). Carrion's disease. I : Behavior of the etiological agent within cells growing or surviving *in vitro*. *Proc. Soc. Exper. Biol. & Med.*, **37**, 587.

RYBINSKY, S. B. (1929). The spontaneous bartonellosis in guinea-pigs. *Rev. Microbiol., Saratov*, **8**. [In Russian, p. 296 ; English summary, p. 358.]

SCHIN, C. (1930). Ueber die *Bartonella muris*. *Jl. Chosen Med. Assoc.*, **20**. [In Japanese, p. 1073 ; German summary, p. 1216.]

SCHWETZ, J. (1934). Sur la présence de certaines inclusions globulaires dans le sang des cobayes et leur ressemblance avec certaines formes de *Bartonella muris ratti*. *Bull. Soc. Path. Exot.*, **27**, 515.

STRONG, R. P., TYZZER, E. E., BRUES, C. T., SELLARDS, A. W., and GASTIABURÚ, J. C. (1915). Report of the first expedition to South America, 1913. Harvard Univ. Press, Cambridge, Mass.

WEINMAN, D. (1935). Les parasites erythrocytaires révélés par la splénectomie : Bartonella et Eperythrozoon. Amédée Legrand, Paris.

——— (1938). *Jl. Infect. Dis.* (In press.)

——— and PINKERTON, H. (1937). Carrion's disease. IV : Natural sources of *Bartonella* in the endemic zone. *Proc. Soc. Exper. Biol. & Med.*, **37**, 596.

WENYON, C. M., and LOW, G. C. (1914). The occurrence of certain structures in the erythrocytes of guinea-pigs, and their relationship to the so-called parasite of yellow fever. *Jl. Trop. Med. & Hyg.*, **17**, 369.

ZINSSER, H., WEI, H., and FITZPATRICK, F. (1937). Agar slant tissue cultures of typhus Rickettsiae (both types). *Proc. Soc. Exper. Biol. & Med.*, **37**, 604.

EXPLANATION OF PLATE III

Fig. 1. *Bartonella tyzzeri* sp. nov. in blood of a splenectomized guinea-pig. Two microscopic fields, *a* and *b*, are shown, each containing one fairly heavily infected erythrocyte. Photomicrograph made from a Giemsa-stained blood-film. (× 1,500.)

Fig. 2. Two clusters (*a* and *b*) of *Bartonella tyzzeri* sp. nov. from a culture in leptospira medium. Clusters composed of rods with a few discs. Photomicrograph of a Giemsa-stained smear. (× 1,500.)

Fig. 3. *a.*—*Bartonella tyzzeri* sp. nov. in a mesenchymal cell surviving in the medium of Zinsser, Wei and Fitzpatrick (see text). The cell infection pattern is indistinguishable from that of *B. bacilliformis.*

b.—*Bartonella tyzzeri* sp. nov. growing extracellularly in the same medium. Drawings from Giemsa-stained smear preparations. (× 1,300.)

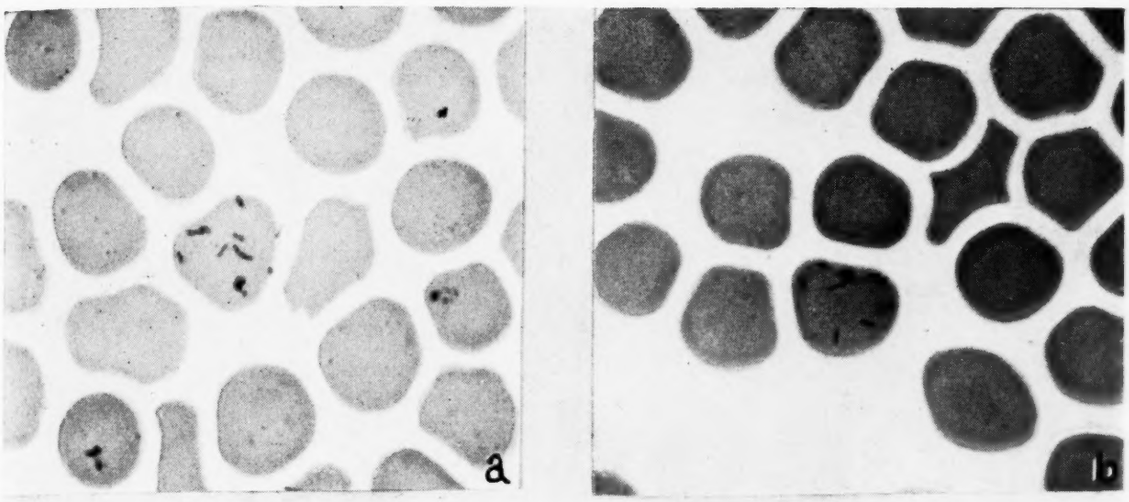


Fig. 1

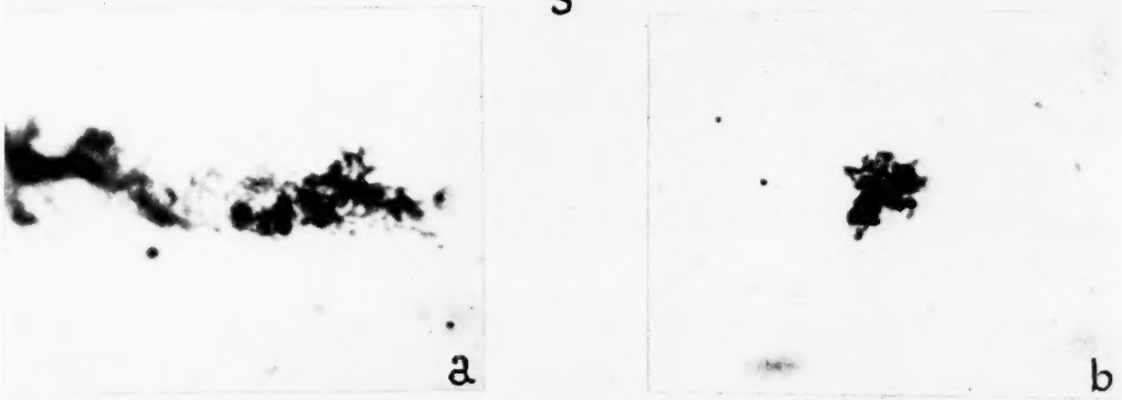


Fig. 2

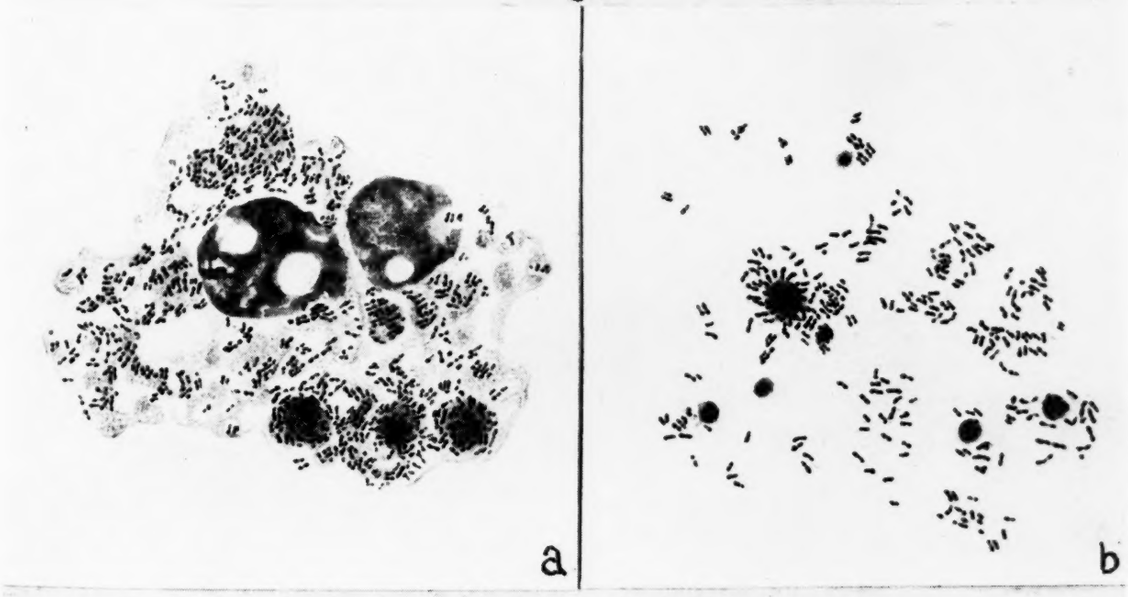


Fig. 3

H. R. Grubb, Ltd., Poplar Walk, Croydon

UNIV. OF MICH.

NEMATODES OF THE REPTILES OF MEXICO.—II

BY

EDUARDO CABALLERO Y C.

(*From the Institute of Biology of Mexico*)

(*Received for publication July 13th*, 1938)

In the present paper we continue the study of the nematodes of the reptiles of Mexico, of which the first part is published in the *Annales de Parasitologie*, Paris.

FAMILY OXYURIDAE COBBOLD, 1864
SUBFAMILY OXYURINAE HALL, 1916
Genus *Ozolaimus* Dujardin, 1845

OZOLAIMUS MEGATYPHLON RUDOLPHI, 1819

Specimens were taken from the caeca of 18 iguanas (*Iguana iguana rhinolopha*) collected in the region of Acapulco, Guerrero, Mexico.

MALE. Length 4·850–5·900 mm.; maximum breadth 0·390 mm. Cuticle with transverse striations. Spicule 1·072–1·189 mm. long by 0·029–0·045 mm. broad at the base.

FEMALE. Length 6·250–7·400 mm.; breadth 0·624–0·721 mm. Amphidelphi uteri; vulva 1·350–2·650 mm. from the posterior end. Eggs 0·135–0·139 by 0·069–0·070 mm., each containing a larva.

HOST: *Iguana iguana rhinolopha*.
HABITAT: Caecum.
GEOGRAPHICAL DISTRIBUTION: Acapulco, Guerrero, Mexico.
TYPE: Collection of Helminthology of the Institute of Biology of Mexico.

OZOLAIMUS CTENOSAURI SP. NOV.

Male and female specimens were taken from the caecum of an iguana (*Ctenosaura acanthura*) from Huajuapan de Leon, Oaxaca, Mexico.

MALE. White, with ends tapering; transparent cuticle with transverse striations; no lateral alae present. Length 4·700–5·187 mm.; maximum breadth 0·370–0·390 mm. Anterior end with two lips measuring 0·029–0·041 mm., with their corresponding papillae. The mouth is continuous with a small vestibule; the oesophagus is 1·560–1·599 mm. in length by 0·057–0·070 mm. in maximum breadth; oesophageal bulb 0·196–0·197 mm. long by 0·168–0·176 mm. broad; anus opens 0·123–0·175 mm. from posterior end. Nerve ring 0·229–0·246 mm. from anterior end, and excretory pore 1·833 mm.

from the same point. Posterior end provided with a pair of alae, 0·164–0·192 mm. in ventral diameter, upheld by a dorsal, chitinous, digitiform process. The accessory piece, 0·094–0·123 mm., has heavy chitinous walls. Papillae consist of a pair of large preanal papillae and another pair of postanal papillae at the end of the caudal process. There is only one spicule, 1·131–1·248 mm. in length by 0·028–0·033 mm. in breadth at the base.

FEMALE. Slightly larger than the male, measuring 8·350–8·450 mm. in length by 0·624–0·702 mm. in breadth, with pointed posterior end. The lips measure 0·041–0·053 mm.; oesophagus 2·632–3·022 mm. long by 0·065–0·078 mm. broad; oesophageal bulb 0·253–0·273 mm. long by 0·253–0·263 mm. broad. Anus opens 0·253–0·273 mm. from the posterior end. Nerve ring 0·351–0·429 mm. from anterior end, and excretory pore 2·613–2·730 mm. from the same end. The vulva is at the hind part of the body, 3·042–3·393 mm. from posterior end, and 2·827–3·100 mm. from anus. Ovejector long, measuring 1·092–1·209 mm. Eggs ovoid, with smooth shell, 0·131–0·135 mm. long by 0·070–0·072 mm. broad.

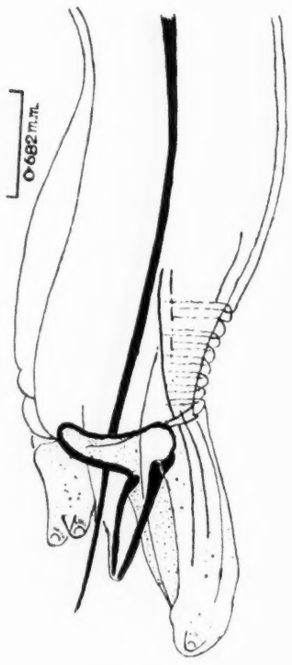


Fig. 1

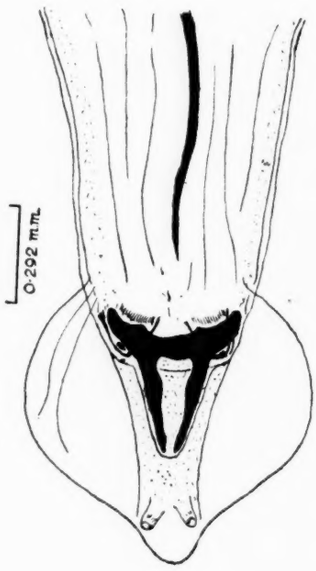


Fig. 2

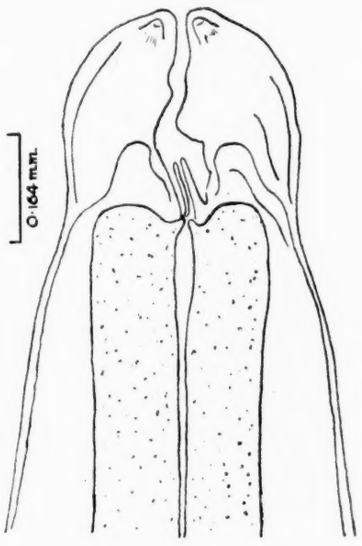


Fig. 3

FIG. 1. Lateral view of the caudal extremity of the male of *Ozolaimus ctenosauri* sp. nov.
FIG. 2. Ventral view of the caudal extremity of the male of *Ozolaimus ctenosauri* sp. nov.
FIG. 3. Ventral view of the anterior extremity of the female of *Ozolaimus ctenosauri* sp. nov.

HOST : *Ctenosaura acanthura.*
HABITAT : Caecum.
GEOGRAPHICAL DISTRIBUTION : Tonauixtla, Puebla, Mexico.
TYPE : Helminthological Collection of the Institute of Biology of Mexico.

Discussion. A comparison of *Ozolaimus megatyphlon* from the caecum of *Iguana iguana rhinolopha* and *Ozolaimus ctenosauri* sp. nov. revealed that the enlargement of the oesophagus characteristic in *O. megatyphlon* never occurs in *O. ctenosauri* sp. nov. We have considered this anatomical character of great interest in the establishment of this new species. Moreover, we have also taken into consideration such other differences as the length of the spicule, the size of the females, and consequently the different measurements of the oesophagus, the location of the excretory pore, etc.

Family HETERAKIDAE Railliet and Henry, 1914
Subfamily Heterakinae Railliet and Henry, 1914
Genus *Strongyluris* Müller, 1894

Strongyluris similis sp. nov.

Four males and six females, taken from the intestine of the ' collar lizard,' *Sceloporus torquatus*, were used for the following description.

Male. White ; 8·900–9·350 mm. long by 0·409 mm. in maximum breadth. Thick smooth cuticle. The anterior end presents many papillae without any special arrangement ; the mouth is surrounded by three lips, on which we find the papillae in the distribution characteristic of the genus. The dorso-ventral diameter is 0·057–0·069 mm. ; the pharynx is 0·213–0·266 mm. long by 0·053–0·061 mm. broad ; the oesophagus is 1·072–1·111 mm. long by 0·061–0·074 mm. in maximum breadth ; the oesophageal bulb is slightly broader than long, measuring 0·214–0·253 mm. in breadth by 0·175–0·273 mm. in length. The anus is subterminal. The nerve ring is located behind the pharynx, 0·468 mm. from the anterior end ; the excretory pore opens a little before the oesophageal bulb, 0·975–1·033 mm. from the anterior end. The caudal extremity, straight and oblique, is wider than the anterior end. It has a caudal ala and a spike. The copulatory organs are formed by the caudal ala, seven pairs of lateral pedunculated papillae and three small ventral ones. The pedunculated papillae are distributed as follows : the first pair, anterior to the anal sucker ; the second pair, which is the longest of all, comprises part of the anterior region of the sucker ; the third, just at the level of the sucker ; the fourth, adanal ; the fifth, postanal, at the same level as the fourth ; the sixth and seventh, further to the end, starting jointly from the same place, at both sides of the spike ; the eighth, preanal, located behind the posterior border of the sucker ; the ninth and tenth, postanal. On the middle line, and on the posterior border of the sucker, there is a little papilla. The preanal sucker has a chitinous ring and measures from 0·074 to 0·086 mm. in diameter. The spicules are subequal and thin ; the largest is 0·780–0·799 mm. long by 0·028 mm. wide at the base, and the small one 0·721–0·780 mm. by 0·028 mm. at the base ; there is no gubernaculum. The relation between the size of the large spicules and the length of the body is 1 : 11·41 to 1 : 11·70.

FEMALE. Length 9·750–10·400 mm.; breadth 0·429–0·487 mm.; the posterior end is broad, oblique, and finely pointed at its extremity. The dorso-ventral diameter of the head is 0·069–0·073 mm.; the pharynx is 0·225–0·253 mm. long by 0·065–0·073 mm. in maximum breadth; the oesophagus is 1·150–1·228 mm. long by 0·065–0·082 mm. broad, and the oesophageal bulb is 0·214–0·273 mm. long by 0·214–0·234 mm. broad. The anus opens 0·195 mm. from the posterior end. The nerve ring is 0·487 mm. from the anterior end, and the excretory pore 0·975 mm. from the same point. The vulva is 3·451 mm. from the posterior extremity. The uterus is divergent, and the ovejector is 0·143 mm. long. The eggs are ovoid, have thick shells, and measure 0·082–0·086 mm. by 0·049–0·053 mm. (measurements made on eggs expelled by the female in physiological serum).

HOST: *Sceloporus torquatus.*
HABITAT: Intestine.
GEOGRAPHICAL DISTRIBUTION: San Angel, Distrito Federal, Mexico.
TYPE: Helminthological Collection of the Institute of Biology of Mexico.
CO-TYPE: U.S. National Museum. Hell. Coll. No.

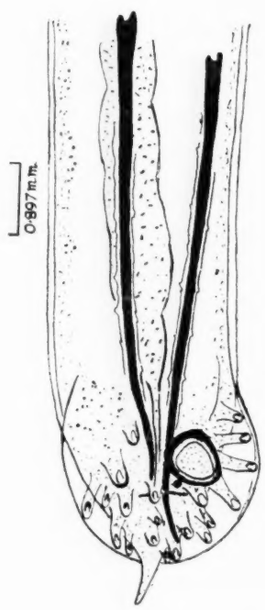


Fig. 4

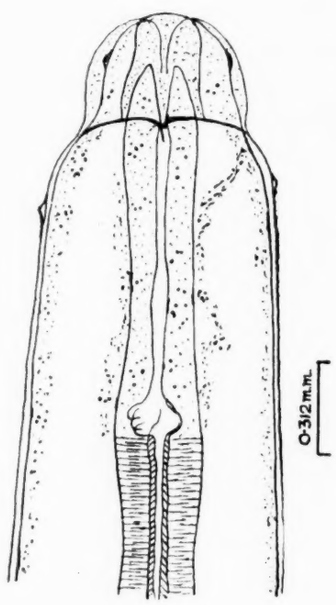


Fig. 5

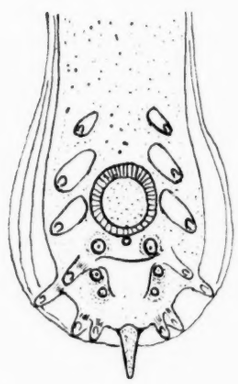


Fig. 6

FIG. 4. Latero-ventral view of the caudal extremity of the male of *Strongyluris similis* sp. nov.
FIG. 5. Lateral view of the anterior extremity of the female of *Strongyluris similis* sp. nov.
FIG. 6. Ventral view of the caudal extremity of the male of *Strongyluris similis* sp. nov.

DISCUSSION. The species described is similar to *S. brevicaudata* in the size of the spicules and eggs and in other anatomical measurements, but differs in the lack of cuticular expansions in the lips, in the relation between the length

of the body and the length of the spicules, and in the absence of papillae in the tail of the female. It is similar to *S. ornata* in the relation between the length of the body and the length of the spicules, but differs in the length of the spicules themselves, in the distribution of the papillae at the caudal end of the male, and in the absence of the pair of papillae at the caudal end of the female. The species differs also from *S. sai* in the arrangement of the papillae at the posterior end of the male, in the relation between the length of the body and that of the spicules, and in the position of the vulva. It differs from *S. media* and *S. rubra* mainly in the relation between the length of the body and that of the spicules.

REFERENCES

CHAKRAVARTY, G. K. (1936). A new nematode *Strongyluris bengalensis* n.sp. with a note on the genus. *Ztschr. f. Parasitenk.*, **8**, 542.

GENDRE, E. (1909). Notes d'helminthologie africaine (deuxième note). *P. V. Soc. Linn. Bordeaux*, **63**, 33.

HARWOOD, P. D. (1935). Notes on Tennessee helminths. II : Two new species of *Strongyluris* (Nematoda) and notes on the genus. *Jl. Tenn. Acad. Sci.*, **10**, 131.

HSÜ, H. F. (1932). A study of some parasitic nematodes from Tonkin, Indo-China, and of *Strongyluris brevicaudata* Mueller, 1894, from Hainan Island, South China. *Peking Nat. Hist. Bull.*, **7**, 99.

——— and HOEPPLI, R. (1931). Parasitic nematodes mostly from snakes collected in China. *Nat. Med. Jl. China*, **17**, 567.

KARVE, J. N. (1938). Some nematode parasites of lizards. Livr. Jub. Prof. Travassos, p. 251. Rio de Janeiro.

PEREIRA, C. (1935). Os *Oxyurata* parasitos de *Lacertilia* do nordeste brasileiro. *Arch. Inst. Biol., S. Paulo*, **6**, 5.

SANDGROUND, J. H. (1933). Parasitic nematodes from East Africa and Southern Rhodesia. *Bull. Mus. Comp. Zool.*, **75**, 263.

——— (1936). Scientific results of an expedition to rain forest regions in eastern Africa. VI : Nematoda. *Ibid.*, **79**, 341.

THAPAR, G. S. (1926). *Ozolaimus* Dujardin, 1845 (= *Macracis* Gedoelst, 1916), a little known nematode genus from the caecum of *Iguana tuberculata*. *Jl. Helminth.*, **4**, 69.

TRAVASSOS, L. (1926). Sobre mais uma especie do genero *Strongyluris* Mueller, 1894, encontrada no Brasil. *Ann. Fac. Med. S. Paulo*, **1**, 83.

FUNGAL INFECTIONS OF MOSQUITOES, ESPECIALLY OF *ANOPHELES COSTALIS*

BY

A. J. WALKER

(*Leverhulme Research Fellow, Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication July 20th*, 1938)

This preliminary paper records the observations made in Sierra Leone of infections of both adult and larval mosquitoes with organisms which, until their fungal nature was recognized, were referred to as ' nematode ova ' and later as ' ovoid bodies.'

REVIEW OF THE LITERATURE

Macfie (1917) reported *Nocardia* and *Fusarium* in a single larva of *Stegomyia fasciata*, the first septate and the second a very fine mycelium with tiny spores. A fungal infection on the surface of laboratory-bred larvae was also noted.

Keilin (1921) described and named as *Coelomomyces stegomyiae* gen. nov., sp. nov. a fungal infection of a single preserved larva of *Stegomyia scutellaris* Walker from a collection of Dr. W. A. Lamborn in Malaya. The size and stages found, as well as the distribution in the larva and the method of development, are all comparable to the material examined in the present study. The cells of the sporangia are clearly figured and described. He observed two types of sporangia, one thick-walled or resting stage, and one thin-walled for immediate reproduction. The spores with which his sporangia were filled were lozenge-shaped, $5\mu \times 1\mu$ in size, and not very numerous. He writes : ' Their shape, however, and the fact that they probably represent the final infective stage of a parasite of an aquatic host suggest that these spores are provided with one or two flagella and are the actual flagellispores or zoospores which are so characteristic of some groups of lower fungi.' As Keilin himself points out, ' It is still necessary to determine (1) the structural character of the mycelium during the early stages of infection . . . ; (2) where conjugation occurs, and at what stage of the development of the parasite ; (3) the structure of the spores ; (4) the mode of their liberation from the body of the host and, finally, (5) the mode of infection of a new host and the formation of the first mycelium.' Moreover, he observes, ' first of all, it is desirable to know if the parasite is confined to but one species of *Stegomyia* or if it infects other species of Culicidae or perhaps other Arthropods.'

Bogoyavlensky (1922) described and illustrated by drawings an infection of the fat-body of *Notonecta* to which he gave the name *Zografia notonectae*. He found this organism in all stages of the insect. His description is quite detailed with regard to the development of the sporangia and of their nuclei.

Eckstein (1922) found near Strassburg about 30 larvae of *Aëdes cinereus*

and *Culicada vexans* out of large numbers of larvae from two particular breeding-places, which were filled with yellowish-brown bodies, 100μ × 60μ in size, and with a thick shell. Such larvae never pupated, and he did not suggest a name for the infection.

Chorine and Baranoff (1929), from Zagreb, described a fungus on the surface of larvae of *A. maculipennis*, and another fungus, belonging to the family of Saprolegniaces, from a *Simulium*, with which they were also able to infect *A. maculipennis* larvae. They suggested that the infection with both these species is produced by motile zoospores, which commence to leave the sporangia after the death of the larva. ' Ces zoospores,' they wrote, ' nagent librement dans l'eau jusqu'à ce qu'elles rencontrent un milieu convenable pour leur développement (c'est-à-dire une larve) et à ce moment, avant qu'elles soient fixées sur une larve, un grand nombre d'entre elles sont mangées par les différents Infusoires.'

Keilin (1927) reviewed Bogoyavlensky's paper and pointed out such close resemblances to *Coelomomyces stegomyiae* that he proposed the name *Coelomomyces notonectae* (Bogoyavensky) in place of *Zografia notonectae*.

Langeron (1929), from Corsica, reported the infection of a single larva of *Culex hortensis* by a Torula type of fungus, and intimated that the infection may have been by way of the tracheae.

Manalang (1930), from the Philippines, described two varieties of what he considered to be coccidia, one brownish-yellow and the other colourless, in 1–2 per cent. of all adult mosquitoes dissected. A single larva of *A. tassellatus* Theo. showed a similar condition in the thorax and first three abdominal segments. The brownish variety measured 45μ × 30μ and had the general appearance of Ascaris eggs. The shell was double-walled with longitudinal ridges, and gave the impression of a coiled larva within. Sections showed undifferentiated granular contents. The smaller, colourless variety was 38μ × 27μ in size, smooth, thick-walled and highly refractile. Light infections were confined to the abdominal cavity, while heavy ones involved the whole body. No coccidia were found in the lumen of the gut. These oöcysts showed no change after being kept in a moist chamber at 37° C. for some weeks. In a footnote Manalang adds that in another district the infection of *A. philippinensis* was so prevalent and so heavy as to cause the death of the mosquitoes.

Roubaud and Toumanoff (1930) experimentally induced an external infection of larvae of *A. maculipennis* with an aspergillus type of fungus. Infection of *Culex* sp. with the same fungus was less successful.

Gibbins (1932), in Uganda, in a series of 18,000 dissections of anophelines, found in the ovaries of one *A. costalis* many objects resembling Ascaris ova. Also very numerous in the ovaries of 6 *A. costalis* and 25 *A. funestus* were ovoid bodies. ' They were light brown in colour, varied from 25μ to 30μ in length, and consisted of a finely granular homogeneous oval body within an outer shell.'

Feng (1933), in Woosung, reported, together with other parasites of mosquitoes, infections, similar to those described by Manalang, in both adults and larvae of *A. hyrcanus* var. *sinensis* and *C. tritaeniorhynchus*. In some ponds larvae were more frequently infected than in others and were easily recognized by their colour and diminished activity. The incidence of infection of the *Culex* larvae was usually higher than of the *Anopheles* larvae. The two types of cysts filled all parts of the larvae except the organs, and appeared in the intestine only when swallowed.

Iyengar (1935), from India, described two fungi occurring in the haemocoele of 7 species of *Anopheles* larvae, and named them *Coelomomyces indiana* sp. nov. and *Coelomomyces anophelesica* sp. nov. Both species live on the fat-body, and fully developed sporangia are found in the third and fourth larval instars. The sporangia have ribbed outer surfaces and show no inner wall. After development a fine filament connects them to the mycelium. *C. indiana* measured 36μ × 60μ and *C. anophelesica* 40μ × 44μ.

FINDINGS IN SIERRA LEONE

The discovery of ' ovoid bodies ' in 4 adult *A. costalis* ♀♀ out of a total night's catch of 4 *A. costalis* ♀♀ and 7 *A. funestus* ♀♀ on November 13th, 1936, stimulated a previously casual interest in these bodies.*

Plate IV, figs. 1, 2, 3 and 4, show the different types of sporangia, which will be referred to as type 1, type 2, type 3, type 4.

Up to the end of March, 1938, there are records of 56 adults, 2 pupae and 544 larvae containing one or other of the above types. They are as follows :

Freetown, 1930–31 : 23 *A. costalis* ♀♀ from 136 caught in the same houses and out of a total of 1,164 dissections. All were apparently type 1. In 20 the ovaries only were involved, and in 3 the whole body.

Kissy, a native village near Freetown, 1930–31 : 9 *A. costalis* ♀♀ out of 1,154 dissections, and 2 *A. funestus* ♀♀ out of 909 dissections. They were chiefly type 1, but at least two are known to be type 2.

Kissy, 1936–37 : 16 *A. costalis* ♀♀ and 1 *A. funestus* ♀ out of approximately 3,000 dissections. Both type 1 and type 2 were encountered, and ovarian infections outnumbered those of the whole body by about 2 to 1.

Kissy and vicinity, 1937 : 1 *A. costalis* ♀ was found among 309 *A. costalis* (♂♂ 36, ♀♀ 273) and 2,843 *A. funestus* (♂♂ 279, ♀♀ 2,564). This infection was limited to the ovaries and has been provisionally classed as type 1.

Among those adults which bred out successfully from some 75 larvae collected in the Kissy district, two *A. costalis* ♀♀ were found to have type 2 in the

*The author's investigations were carried out during the period November, 1936, to July, 1938. All records previous to November, 1936, have been supplied by Professor T. H. Davey, from unpublished figures obtained in connection with a mosquito-survey carried out by Gordon *et al.* (1932) ; the Kissy results, 1936-37, represent combined investigations by Professor Davey and the author.

ovaries. These larvae were not examined before pupation, and all the emerging adults were maintained in one cage for 7 days.

Two *A. costalis* ♂♂ were bred from pupae collected from the same pools in which infected larvae were also found. One was infected with type 4 and the other with type 3.

Two pupae of *A. costalis* developed from larvae previously known to be infected with type 3.

Five hundred and thirty-seven larvae showing either mycelium alone or type 3 or type 4 were found in some 1,500 examined from the vicinity of Kissy. From a lot of 8 obtained in Freetown, 5 contained type 4.

One *A. funestus* larva out of 24 anopheline larvae received from Dr. F. H. C. Watson, Marampa, was filled with type 3.

One *A. costalis* larva from an unrecorded number received from Bo also contained type 3.

Adults. With the exception of the two above-mentioned males, no further adults have developed from larvae known to be infected, although the majority of them were kept until they died. Non-infected larvae from the same catches developed successfully in the same proportion as larvae obtained from other sources or bred from eggs laid in the laboratory. From this small number of adults we learned that light infections in the female were almost always limited to the ovaries, in which case egg-development was wholly inhibited. Where the infection spread throughout the entire body it was so severe as to cause the death of the host. The two females bred from larvae collected from the pool, which later was known to produce infected larvae repeatedly, were not examined in the larval state. After emergence, they were maintained 6 and 13 days respectively in a cage with other bred mosquitoes, both ♂♂ and ♀♀, and were fed on glucose solution and human blood. The stage at which they became infected is not known. The two males were obtained from two different pools, both of which contained infected larvae at the time. Both failed to emerge completely from the pupal case. In one, a few sporangia of type 4 were seen in the head and thorax, but the dissection was a poor one and the infection may have been more widespread. Under the dissecting microscope, the other male showed a loose, greyish, granular mass just anterior to and about half the size of the testes. A small localized infection was suspected. Crushed under a cover-glass, remnants of mycelium remained visible for some minutes, and more than 100 type 3 sporangia were seen. These did not present a cupped appearance until Gater's fluid was added.

Pupae. Two pupae were found dead before their metamorphosis was complete and some degeneration had taken place. One was dissected and showed well-developed sporangia of type 3 in the thoracic portion. The other was sectioned and type 3 sporangia were found distributed throughout the body. There were a few scanty threads of mycelium in the abdominal portion.

Larvae. The existence of these bodies in larvae was not suspected until March, 1937, when, at the request of the late Dr. A. M. Evans, 30 larvae of

A. costalis were mounted on slides in De Faure's medium and 7 of these were found to be filled with type 3. At a later date, when investigating the pool which most constantly contained infected larvae, we found that a large proportion of the 83 examined were infected. In this material we were able to observe earlier stages of the infection. From another pool, all stages of larvae, even the very tiny ones, contained sporangia.

TECHNIQUE.—Dissections were made in tap-water or normal saline. The sections were cut from material fixed in hot Bles's fluid and stained with haemalum and eosin. Whole larvae, ovaries and sporangia were mounted in De Faure's medium or in Gater's fluid, and sealed with gold size or gutta-percha composition.

Bles's fluid

70 per cent. alcohol	90 c.cm.
Formaldehyde	7 c.cm.
Glacial acetic acid	3 c.cm.

De Faure's medium

Gum arabic	30 gm.
Chloral hydrate	50 gm.
Glycerine	20 c.cm.
Distilled water	50 c.cm.
Cocaine hydrochloride	0·5 gm.

Filter

Gater's modification of Berlese's fluid

Distilled water	10 c.cm.
Gum arabic	8 gm.
Chloral hydrate	70 gm.
Glycerine	5 c.cm.
Glacial acetic acid	3 c.cm.

Dissolve in water-bath at 80° C. Filter through 3–4 thicknesses of muslin or with suction.

Heavy infections in living specimens of larger larvae are readily recognized by the naked eye or with the aid of a hand-lens. Scanty infections can only be detected under the microscope. The same larva may be examined repeatedly, or many may be examined together in a short time, by using the simple apparatus illustrated in the accompanying diagrams. Because of the varying amount of pigment on the upper surface of the larvae it is preferable to view them from

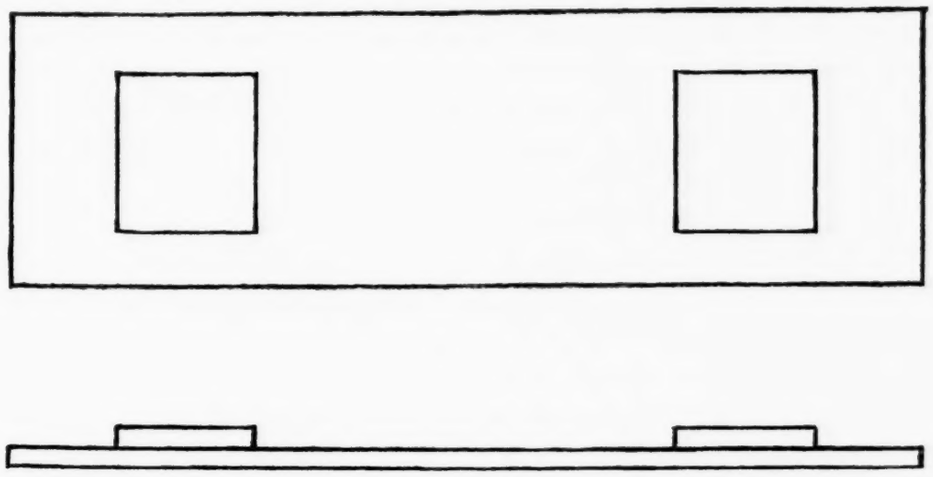


DIAGRAM 1

below, if the contents of the body-cavity are to be visible. The larva is placed in water, in the depression of a hollow-ground slide under a cover-slip, and inverted on the two small glass squares fastened with balsam near each end of an ordinary glass slide.

In this way, the specimen is less likely to be damaged, the under surface of the lower slide remains dry and does not stick to the microscope platform, and it is not necessary to dry the upper slide thoroughly, as the surface area of the small squares is negligible. The cavity of the hollow-ground slide is much too large for very small larvae, as it is impossible to keep them in the field. A small cell was therefore made by cutting a circular opening in a large drop of gutta-percha composition and covering it with a cover-glass.

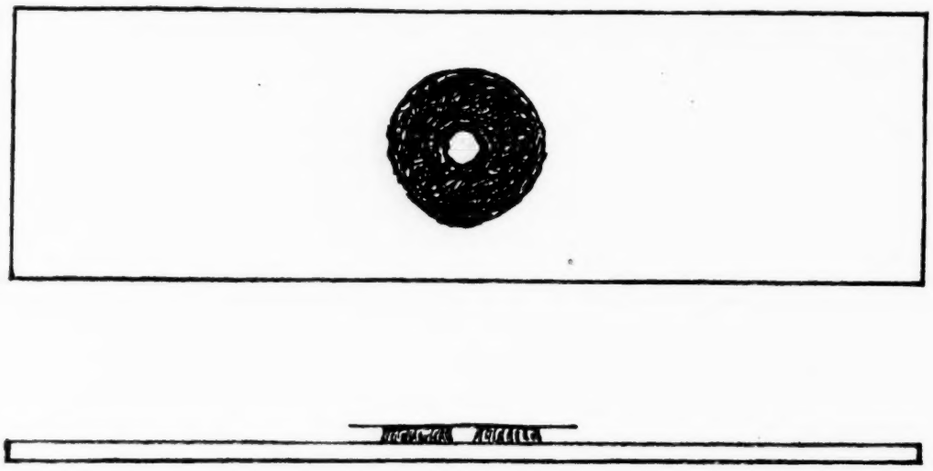


DIAGRAM 2

Using the stereoscopic attachment with the binocular microscope, and directing particular attention to the relatively clear areas between the abdominal segments, it was easy to see branching filaments of a non-septate mycelium. In larvae with few sporangia, these branching hyphae resembled coral growing in water, the free portions moving to and fro with each pulsation of the coelomic fluid. The mycelium was closely adherent all around the wall of the intestine, with the free ends directed outwards, and was evidently more numerous and more compact in the region of the intestinal caeca and less so towards the posterior portion of the gut. Occasionally there was also a peripheral distribution, with the loose portions protruding inward. This arrangement was also confirmed by the examination of sections. After a variable period of time, some of the terminal hyphae appeared to become swollen and rounded, and then, almost without warning, the whole larva seemed to become filled with yellow sporangia, and individual hyphae could no longer be recognized. Free sporangia could be seen moving with the current of the coelomic fluid in all parts of the larva, head, anal gills, etc.

The mycelium varies from 3μ to 8μ in thickness, and a number of densely staining nuclei are seen in the individual hyphae. The same is true of the form-

ing and fully developed sporangia, some of which reveal evenly distributed and evenly spaced nuclei, with a proportionate amount of cytoplasm, which stains more or less densely. Others show scanty groups or irregularly placed collections of nuclei. One larva may show mycelium only, another mycelium and sporangia, while yet another may have many sporangia and either scanty mycelium or none at all. As yet we can distinguish the type of infection only after the sporangia have been formed. Types 1 and 4 measure between 45μ and 60μ in length, and types 2 and 3 from 25μ to 35μ. As yet, only type 1 and type 2 have been encountered in wild-caught adults, and type 3 and type 4 regularly in larvae. Only one larva has been found to contain more than one type of sporangia. This was a fourth stage larva of *A. costalis*. It was packed with type 4, and on closer examination compact masses of type 3 were to be seen in the distal part of the mid-gut and in the dilatation of the hind gut. This larva possibly became infected in the usual way with the larger form, and then ingested large numbers of the smaller. One larva containing type 3 was treated with KOH before mounting, and the crenation of the surface of the sporangia was no longer apparent.

Apparently the physical presence of large numbers of sporangia—the thorax is usually packed full, while the number diminishes towards the tail (Plate IV, figs. 5, 6)—is sufficient to prevent the development of the larvae and to account for their death.

Numerous attempts were made to culture all types of sporangia and two kinds of mycelium on various artificial media. Some were killed by overgrowth of bacteria, while others grew various moulds which resembled the usual laboratory contaminants more than anything in mosquitoes. Sporangia were kept in water for over a year. At first it was thought that some organization was taking place within them, but, after cracking the shell with pressure, the contents dissolved and disappeared in practically the same way as they did when fresh.

Eggs, fresh larvae, pupae, etc., were similarly placed in water containing sporangia, either intact or ground up after drying, or material from infected larvae or living infected larvae; but the results were negative. Larvae have been shown to ingest sporangia in large numbers, and to pass them out apparently unchanged. The following rather crude experiments, however, may have some significance.

One particular breeding-place (field 2), which had consistently produced infected larvae practically throughout the year, was flooded by irrigation after it had been almost completely dry for two days. No larvae of any kind were visible. A very fine-mesh copper sieve with metal sides was placed in the water, half submerged, but floating by means of large corks attached to it. The next day 21 medium-sized laboratory-bred *A. costalis* larvae were placed within the sieve. By this time numerous tiny larvae, evidently from eggs laid in the moist earth, were apparent in the pool. After one day only 8 larvae could be found

in the sieve. Four of these showed no infection on microscopic examination and were returned to the pool on the following day. On the second day two of those remaining were examined and no infection was recognized. Only three larvae remained in the sieve on the fourth day and two of these were heavily infected with type 3, as were also 66 per cent. of the 162 smaller larvae subsequently collected from other parts of the pool. It should be pointed out that from field 2 only type 3 has so far been recovered.

A cement tank was then constructed in the laboratory grounds, where conditions of shade, sun, etc., resembled those of field 2, which was three miles distant. Some 30 gallons of water, sediment and vegetation were transferred from field 2 to this tank, and all the larvae which could be seen were removed. Of these, 10 in all, two were infected with type 3. Seventy-five 2-3-day-old laboratory-bred *A. costalis* larvae were added. Two days later 100 fresh larvae were added. On this occasion one well-grown infected *A. costalis* larva, which had obviously escaped detection, was recovered. During the ensuing three days, 12 larvae, proportionate in size to the period in which fresh larvae had been added, were found to contain either mycelium only or both mycelium and type 3 sporangia. Almost all the negative larvae pupated successfully.

Twenty-eight days after the tank was thus filled, 1,000 young larvae and some 600 eggs of *A. costalis* were placed in it. No infected larvae were subsequently found and none of the high proportion of adults which emerged showed any trace of infection.

The tank was then cleaned and filled with 30 gallons of clear water from the brook, well below the point where the irrigation was led off. Some 900 larvae and 400 eggs gave an equally high yield of adults. There were no infections.

Up to the point where this investigation was suspended, on account of the drying-up of the streams, only *A. costalis* and rarely *A. funestus* appeared to suffer, although many other species were examined. On one or two occasions *Culex* sp. larvae were found to contain a small number of sporangia, but in each instance these were traced to the gut.

DISCUSSION

When analysing these findings and those of other workers, it should be kept in mind that some investigators were dealing with infections of the exterior of larvae, while others were studying internal infections.

Roubaud and Toumanoff, for instance, used a known fungus to infect larvae externally. It would seem that Macfie and Chorine and Baranoff were dealing with one of the natural fungal infections of the outer surface of mosquito larvae ; and it is probable that Langeron's fungus was of a similar kind.

In common with the remaining investigators cited in this paper, we, on the other hand, have been studying an infection of the interior of mosquitoes. The internal infection described by Macfie in no way resembled ours. Eckstein was probably dealing with a large type of a related species, and his description

of the general appearance and movements of the infected larvae, as well as their fate, is consistent with our observations. It would appear that Gibbins was referring to similar or perhaps identical infections in Uganda, and it is probable that Manalang and Feng were working with a variant of the same infection, perhaps comparable to those of Iyengar.

One of our types corresponds in size and somewhat resembles that of Keilin, but we have not yet been able to observe as much detail in the internal structure of the sporangia. Under gentle pressure, fresh and stored sporangia have been ruptured. Sometimes an oval body with a soft limiting membrane has emerged intact, but usually the contents flow out and dissolve immediately. The answers to Keilin's questions would clarify many points, especially those regarding the possibility of the practical application in mosquito control. Bogoyavlensky's results are interesting in that they show a similar infection in another aquatic insect.

In a personal communication to Professor R. M. Gordon, Sir Rickard Christophers suggested that the infections here described were similar to those of Keilin but of a different species.

Iyengar's findings are of very great interest. Seven species of anophelines are known to be infected, and his are the first definite fungal infections of anopheline larvae reported. We have not as yet seen the filaments remaining after the sporangia are formed and connecting them to the parent mycelium, nor the longitudinal or circular ribbing of the shell of the sporangia. His mycelium is remarkably thick, and the predilection for the fat-body has not been observed in our specimens.

The organism or organisms reported in the present paper clearly belong to the genus *Coelomomyces*, but, as apparently they are not identical with any of the species described and named by Keilin (1921, 1927) and by Iyengar (1935), the name *Coelomomyces africanus* sp. nov. is proposed for the commonest of the forms found in *A. costalis* described in the present paper, i.e., type 3.

SUMMARY

A preliminary report on fungal infections of mosquitoes in Sierra Leone records their presence in 53 adult *A. costalis*, 3 adult *A. funestus*, 2 *A. costalis* pupae, 543 *A. costalis* larvae, and 1 *A. funestus* larva.

Two adult infected males emerged from pupae obtained in breeding-places in which numerous infected larvae were also found.

The infection in females occurs most frequently in the ovaries and causes sterilization. General infections are always fatal.

Less than 1 per cent. of infected larvae develop to maturity.

Four types of sporangia were observed, and microphotographs of these are appended. The appearance, development and distribution of mycelium and sporangia are briefly described and illustrated.

One larva showed one type of sporangia in the body cavity and a different one in the lumen of the gut.

The crenated appearance of the surface of one type of sporangia appeared to be lost when the whole larva was treated with potassium hydroxide before mounting.

Attempts to grow mycelium and sporangia on artificial media have as yet been unsuccessful.

Clean larvae failed to develop an infection when fed on sporangia or when maintained in various preparations of infected material.

Infection developed in two of three larvae remaining out of 21 laboratory-bred specimens originally isolated in a sieve floating in one special breeding-place.

Within a few days, a small number of laboratory-bred larvae also became infected when placed in water, sediment, etc., from the same pool. But after some time had elapsed no new infections appeared, although large numbers of fresh larvae were added. Attempts to infect larvae in water from a nearby brook were also unsuccessful. It is conceivable that the source of infection is present in the water but does not survive for long.

Various reported infections of mosquitoes are cited and compared with the forms here described, and it would appear that all may be related to those described by Keilin (1921) and Iyengar (1935).

The name *Coelomomyces africanus* sp. nov. is proposed for the type species in this group.

With four exceptions, all infections were found in *Anopheles costalis*, one of the most important vectors of malaria in West Africa.

ACKNOWLEDGEMENTS.—The writer gratefully acknowledges the interest taken in this work, and the assistance given, by Colonel Sir S. Rickard Christophers, London School of Hygiene and Tropical Medicine; Dr. E. C. Smith, Medical Research Institute, Lagos, Nigeria; the staff of the Institut für Schiffs- und Tropenkrankheiten, Hamburg, Germany, and particularly Professor Dr. E. Martini (Entomology), Professor Dr. E. Reichenow (Parasitology), and Professor Dr. E. G. Nauck (Pathology). All the microphotographs, except fig. 6, Plate IV (which was made in the Medical Research Institute, Lagos, Nigeria), have been taken in the Institut für Schiffs- und Tropenkrankheiten, Hamburg, Germany.

REFERENCES

BOGOYAVLENSKY, N. (1922). *Zografia notonectae* n.g., n.sp. *Arch. Soc. Russe Protist.*, **1**, 113. [In Russian; Germany summary, p. 118.]

CHORINE, V., and BARANOFF, N. (1929). Sur deux champignons parasites d'*Anopheles maculipennis* Mg. *C. R. Soc. Biol.*, **101**, 1025.

CHRISTOPHERS, SIR S. R. (1937). Parasites of mosquitoes. [In manuscript.]

ECKSTEIN, F. (1922). Beiträge zur Kenntnis der Stechmückenparasiten. *Zentralb. f. Bakt., Orig.*, **88**, 128.

FENG, LAN-CHOU (1933). Some parasites of mosquitoes and flies found in China. *Lingnan Sci. Jl.*, **12**, *Suppl.*, 23.

GIBBINS, E. G. (1932). Natural malaria infection of house-frequenting *Anopheles* mosquitoes in Uganda. *Ann. Trop. Med. & Parasitol.*, **26**, 239.

GORDON, R. M., HICKS, E. P., DAVEY, T. H., and WATSON, M. (1932). A study of the house-frequenting Culicidae occurring in Freetown, Sierra Leone. *Ibid.*, **26**, 273.

IYENGAR, M. O. T. (1935). Two new fungi of the genus *Coelomomyces* parasitic in larvae of *Anopheles*. *Parasitol*, **27**, 440.

KEILIN, D. (1921). On a new type of fungus, *Coelomomyces stegomyiae* n.g., n.sp., parasitic in the body-cavity of the larva of *Stegomyia scutellaris* Walker (Diptera, Nematocera, Culicidae). *Ibid.*, **13**, 225.

——— (1927). On *Coelomomyces stegomyiae* and *Zografia notonectae*, fungi parasitic in insects. *Ibid.*, **19**, 365.

LANGERON, M. (1929). Mycose d'une larve de *Culex hortensis* de Corse. *Ann. Parasitol.*, **7**, 107.

MACFIE, J. W. S. (1917). Fungal infections of mosquito larvae. *Rep. Accra. Lab.*, 1916, Section E, 7, p. 76.

MANALANG, C. (1930). Coccidiosis in *Anopheles* mosquitoes. *Philipp. Jl. Sci.*, **42**, 279.

ROUBAUD, E., and TOUMANOFF, C. (1930). Essais d'infection expérimentale de larves de Culicides par quelques champignons entomophytes. *Bull. Soc. Path. Exot.*, **23**, 1025.

EXPLANATION OF PLATE IV

Fig. 1. Large smooth type of sporangium (type 1) in water. (× 500.)

Fig. 2. Small smooth type of sporangium (type 2) in water. (× 500.)

Fig. 3. Small crinkled type of sporangium (type 3) in De Faure's medium. (× 500.)

Fig. 4. Large crinkled type of sporangium (type 4) in De Faure's medium. (× 500.)

Fig. 5. *A. costalis* larva showing head, thorax, first and terminal segments packed with type 3 sporangium, in De Faure's medium. (× 67.)

Fig. 6. *A. costalis* larva with type 4 sporangium in De Faure's medium. (× 95.)

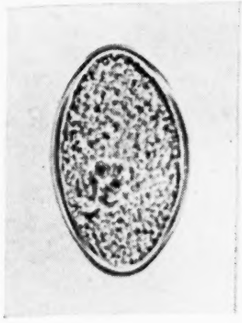


Fig. 1

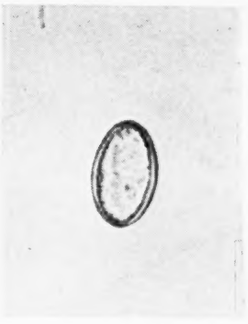


Fig. 2

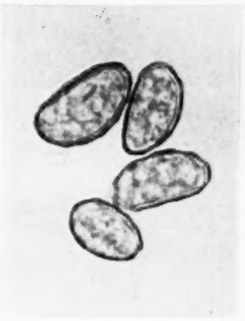


Fig. 3

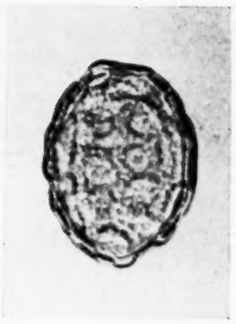


Fig. 4

Fig. 5

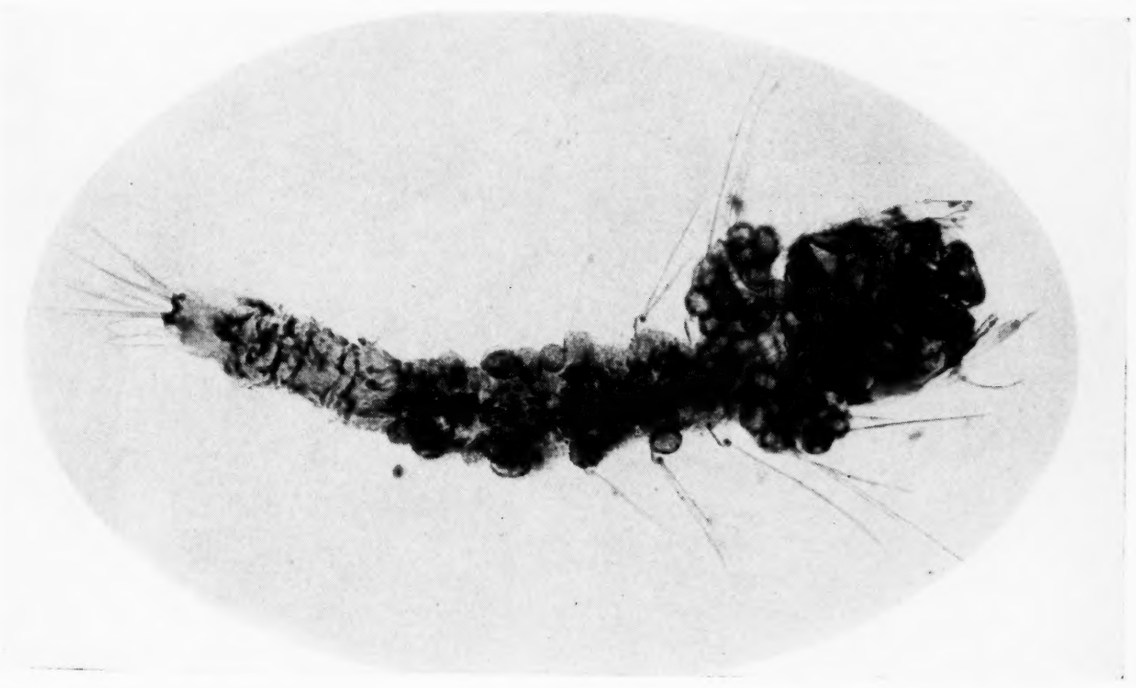


Fig. 6

H. R. Grubb, Ltd., Poplar Walk, Croydon

UNIV. OF MICH.

EXPLANATION OF PLATE V

Fig. 1. Cross-section of a larva stained with haemalum-eosin, showing branching of mycelium, irregular distribution of the nuclei in the hyphae and sporangia, and an empty sporangial shell of type 3. (× 900.)

Fig. 2. Cross-section of larva stained with haemalum-eosin, showing branching of mycelium, swelling of terminal portions and sporangia of type 4. (× 260.)

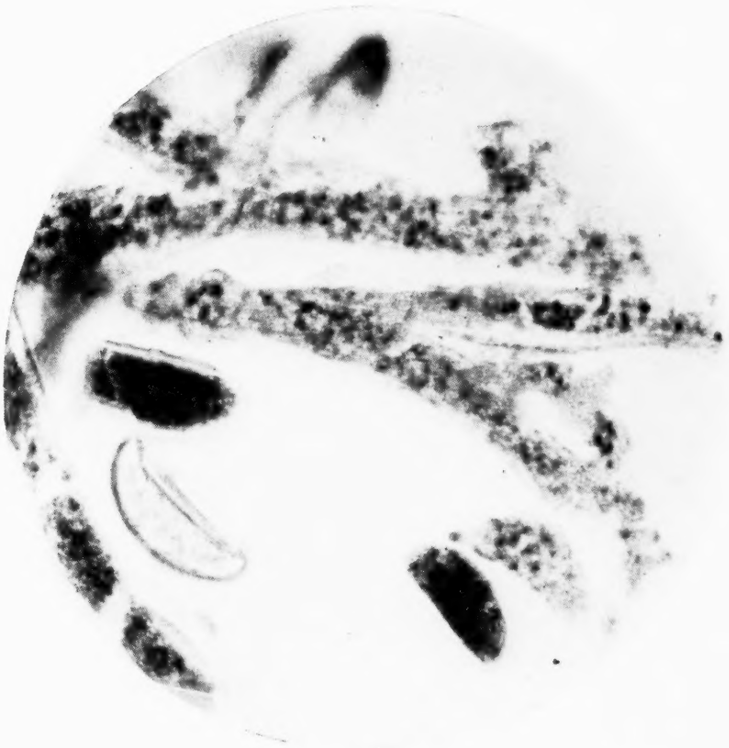


Fig. 1

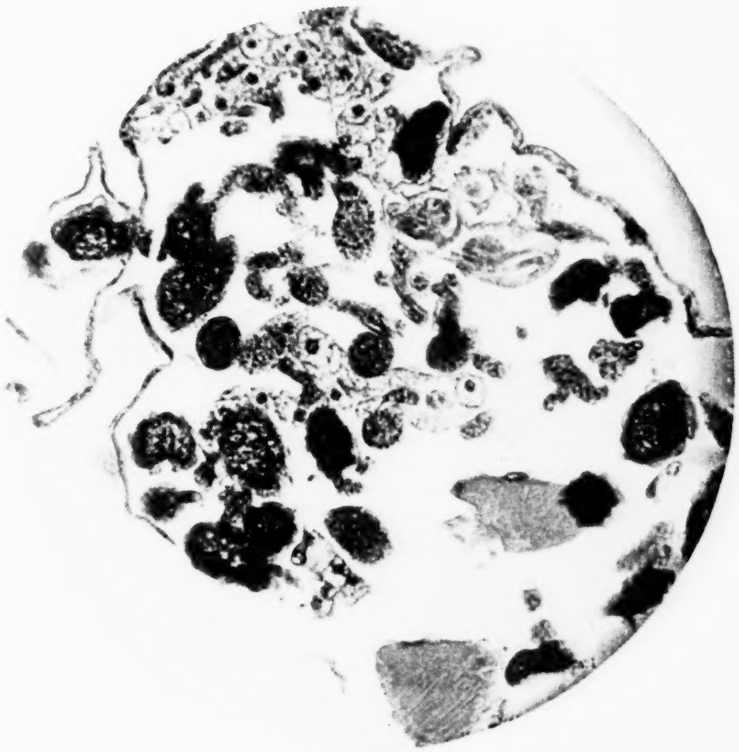


Fig. 2

H. R. Grubb, Ltd., Poplar Walk, Croydon

UNIV. OF MICH.

EXPLANATION OF PLATE V

Fig. 1. Cross-section of a larva stained with haemalum-eosin, showing branching of mycelium, irregular distribution of the nuclei in the hyphae and sporangia, and an empty sporangial shell of type 3. (× 900.)

Fig. 2. Cross-section of larva stained with haemalum-eosin, showing branching of mycelium, swelling of terminal portions and sporangia of type 4. (× 260.)

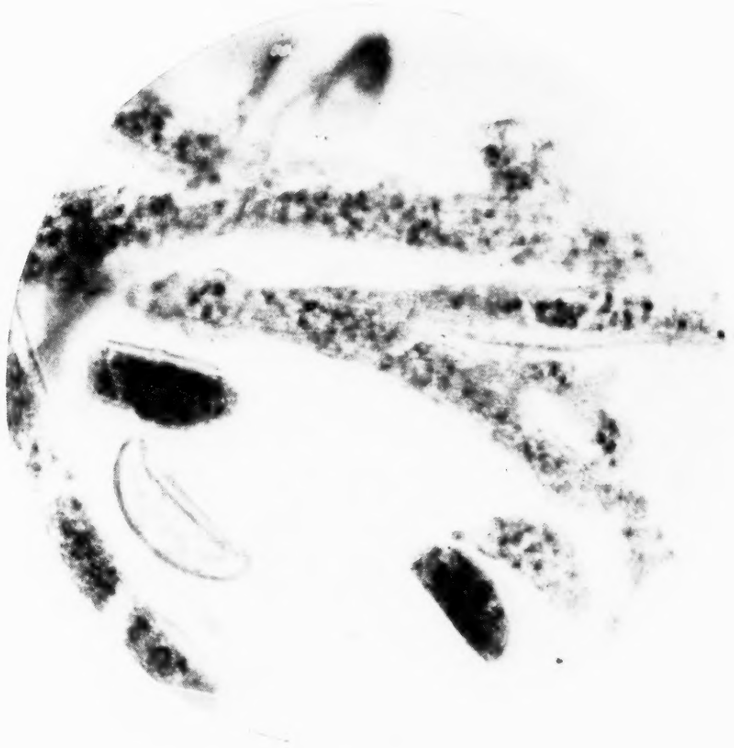


Fig. 1

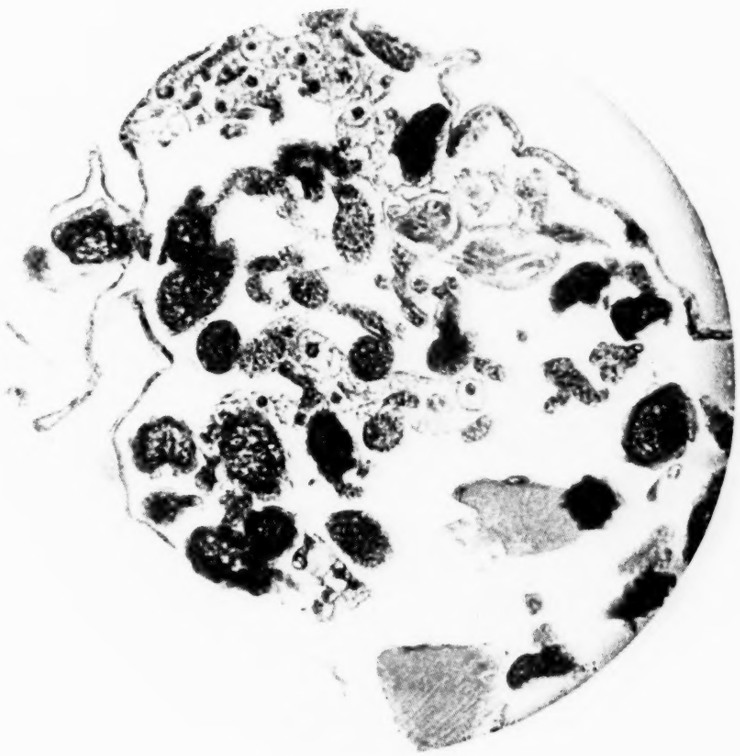


Fig. 2

H. R. Grubb, Ltd., Poplar Walk, Croydon

UNIV. OF MICH.

A THIRD NOTE ON A STRAIN OF *TRYPANOSOMA GAMBIENSE* TRANSMITTED BY *GLOSSINA MORSITANS*

BY

J. F. CORSON

(*Medical Officer at Tinde Laboratory, Tanganyika Territory*)

(*Received for publication August 6th*, 1938)

In March, 1934, a strain of *Trypanosoma gambiense* was isolated from man in Uganda; it was received at Tinde Laboratory in monkey 1292 on August 3rd, and transmissions with *Glossina morsitans* were begun in September, 1934. Observations on the strain from that time until February, 1937, were recorded in two papers (Corson, 1936, 1937), and the present note is a continuation of them.

The object of the experiment was to see whether this strain, by continued cyclical passages through *G. morsitans*, or in the course of mechanical transmissions, would come to resemble *T. rhodesiense*. It was found that, although some resemblance was shown in a few inoculated guinea-pigs and white rats, the changes were confined to a few animals and were not continued in sub-inoculated animals. Transmissions by *G. morsitans* were made from monkey to monkey only, and no changes in the characters of the strain were found; virulence and morphology, however, were the only characters investigated.

Since February, 1937, three more passages by *G. morsitans* have been made, viz., from monkey 76 to monkey 110, from monkey 75 to monkey 115, and from monkey 115 to monkey 133. So far (up to June, 1938) the cyclically transmitted strain appears to have undergone no change. Two rats and a guinea-pig, inoculated from monkey 115, and two rats, inoculated from monkey 133, did not show infection.

Direct transmissions through monkeys, guinea-pigs and white rats, more or less in rotation, have been continued, with somewhat varying results. In monkeys and in some guinea-pigs and rats the strain retained its low pathogenicity, but in a rat and a guinea-pig which were inoculated in April, 1937, from rat 1948 the strain showed much greater virulence, and this continued for rats and guinea-pigs, though not for monkeys; a goat showed a transitory infection, and a sheep resisted completely when inoculated with these trypanosomes of increased virulence for guinea-pigs and rats. The strain also became less polymorphic, nearly all trypanosomes being somewhat slender, with a rather short free flagellum and very rarely showing any nuclear displacement, even in swarming infections. There was no real resemblance to any Tanganyika strains of human trypanosomes that have come under my observation. Four attempts to infect *G. morsitans* from monkeys and guinea-pigs of this direct transmission line failed.

Tables I and II give lists of most of the animals.

TABLE I

Monkeys (*Cercopithecus*) infected by *G. morsitans*

Monkey	Infected from monkey	Alive on June 30th ; days	Died on	Length of life in days	Cerebrospinal fluid		Remarks
					Living tryps.	Leuco-cytes	
1292	1282		2.5.36	750	80	230	Infected by *G. palpalis*
15	1292		27.6.36	630			
17	13		18.11.36	700	100	1,600	Chloroformed
18	1292		20.1.37	837	500	800	,,
14	18		27.8.37	270	0	268	Necrosis of jaw ; chloro-formed
20	17		23.7.37	889	16	2,800	Chloroformed
30	20		15.3.38	925	0	1,400	,,
36	30		16.3.38	808	0	96	,,
32	30	977					
33	32	938					
35	32	899					
40	36	858					
43	35	847					
45	33	809					
48	40	789					
54	45	745					
53	40		18.3.38	639	0	35	Fractured leg ; chloro-formed
75	48	576					
76	54	570					
115	75	537					
110	76	501					
133	115	88					

Note. Trypanosomes are still present in the blood of the monkeys.

TABLE II
Animals of the direct transmission branch

Animal	Inoculated from	Character of infection	Length of life, in days	Remarks
r. 1869	m. 1292	chronic	230	
r. 1871	r. 1869	...	186	
r. 1871a	...	...	105	Paralysis of hind legs
r. 1873	...	...	62	Cause of death unknown
m. 55	...	...	301	
gp. 371	m. 55	semi-acute	107	Posterior-nuclear forms
r. 1907	gp. 371	acute	69	
r. 1907a	...	chronic	98	Paralysis of hind legs
r. 1917	...	...		Died of abdominal abscess
r. 1917a	...	...		Died of septic peritonitis
m. 71	...	...	354	
gp. 392	...	acute	93	Posterior-nuclear forms
gp. 400	r. 1907	semi-acute	193	„ „ once
m. 73	...	chronic	269	
r. 1939	gp. 400	...	149	
r. 1939a	...	...	346	Recovered ?
gp. 405	m. 73	?	23	Probably died of epidemic disease
gp. 410	...	semi-acute	80	
r. 1938	gp. 405	chronic	172	
r. 1938a	...	...	157	
r. 1947	gp. 410	...	267	
r. 1947a	...	...	188	Died of abscess of lung
r. 1918	gp. 392	acute	111	
r. 1918a	...	chronic	74	
m. 78	r. 1918	...		Alive 496 days (June 30th, 1938)
m. 79	...	...	143	Died of peritonitis
r. 1950	...	...	329	
r. 1950a	...	...	80	
m. 80	...	...		Alive 494 days (June 30th, 1938)
gp. 422	m. 80	acute	75	
gp. 421	...	semi-acute	98	
r. 1977	gp. 422	chronic	67	
r. 2013a	gp. 421	...	133	
r. 2013	...	...	125	
m. 102	r. 2013	...	103	Died of bacillary infection
gp. 450	m. 102	...	175	
m. 114	gp. 450	...		Alive 241 days (June 30th, 1938)
r. 2081	...	...	88	
r. 2081a	...	...	97	Cause of death unknown
r. 1948	m. 78	chronic	124	
r. 1976	r. 1948	acute	28	
m. 87	...	semi-acute	87	
gp. 432	m. 87	...	140	
r. 2025	gp. 432	acute	28	
r. 2025a	...	...	28	
gp. 426	r. 1948	...	57	
m. 63	gp. 426	semi-acute	164	
r. 1998	m. 63	acute	19	

r. = rabbit ; m. = monkey ; gp. = guinea-pig.

TABLE II—*Continued*

Animal	Inoculated from	Character of infection	Length of life, in days	Remarks
r. 1998a	...	...	22	
r. 2012a	...	...	26	
r. 2012	...	...	27	
gp. 435	r. 2012	...	60	
r. 2021	gp. 435	acute to chronic	103	
r. 2021a	...	acute	34	
r. 2036	...	...	17	
r. 2036a	...	...	27	
m. 101	...	acute to chronic		Alive 345 days (June 30th, 1938)
Goat 23	r. 2021	transitory ?		Alive 323 days ; trypanosomes seen only twice
Sheep 202	...	negative		Alive
gp. 482	goat 23	...		
gp. 482a	...	...		
r. 2035	m. 101	acute	29	
r. 2035a	...	...	24	
gp. 446	r. 2035	acute	77	
r. 2063a	gp. 446	...	29	
r. 2063	...	...	37	
r. 2071a	...	...	34	
r. 2071	...	...	31	
m. 112	...	chronic		Alive 262 days (June 30th, 1938)
r. 2080	m. 112	acute	14	
r. 2080a	...	...	16	
m. 113	r. 2071	chronic		Alive 242 days (June 30th, 1938)
r. 2092	m. 113	acute	19	
r. 2092a	...	...	25	
gp. 485	...	?	39	Not sufficiently examined
gp. 472	...	semi-acute	102	
r. 2123	gp. 472	?	14	Not sufficiently examined
r. 2123a	...	?	91	
r. 2176	...	semi-acute	103	
gp. 497	...	?	77	Not sufficiently examined
r. 2106	r. 2092	acute	21	
r. 2106b	...	semi-acute	141	

r. = rabbit ; m. = monkey ; gp. = guinea-pig.

COMMENT

The chief point of interest seems to be the long life of the monkeys after infection by *G. morsitans* ; there is no reason to think, however, that transmission by *G. morsitans*, instead of by *G. palpalis*, had any effect on the pathogenicity of the strain. Other points of interest perhaps are the tendency to adaptation to certain species of host, and the great individual differences in resistance between animals of the same species.

REFERENCES

CORSON, J. F. (1936). Experimental transmission of *Trypanosoma gambiense* by *Glossina morsitans* through monkeys. *Ann. Trop. Med. & Parasitol.*, **30**, 389.

——— (1937). Further observations on a strain of *Trypanosoma gambiense*. *Ibid.*, **31**, 275.

A NOTE ON INTRACORPUSCULAR METHAEMOGLOBIN IN PLASMOCHIN TOXICITY*

BY

HENRY FOY

AND

ATHENA KONDI

(*Malaria Research Laboratory, Refugee Hospital, Thessaloniki, Greece*)

(*Received for publication June 29th*, 1938)

The constant occurrence of pseudo-methaemoglobin† in blackwater fever in Macedonia (Fairley and Bromfield, 1937; Foy and Kondi, 1938), which had been mistaken by all previous observers for true methaemoglobin, led to the question of what pigment was present in cases of plasmochin toxicity, and in the blackwater said to follow the administration of plasmochin simplex.

The cyanosis characteristic of plasmochin poisoning has always been attributed to the presence of intracorpuscular methaemoglobin, giving rise to a prominent absorption band in the red region of the spectrum above 600 $m\mu$. As, however, a similar band in blackwater fever had been erroneously described as methaemoglobin, it seemed desirable to ascertain the exact nature of the substance which produces the absorption band in the red in cases of plasmochin toxicity; the following note and spectrogram have reference to this problem.

Although we have carried out a large number of treatments with the atebrin-plasmochin combination, we have rarely seen an unquestioned case of cyanosis accompanied by the presence of abnormal pigments in the blood-stream when normal doses have been given (atebrin 0·3 gm. plus plasmochin 0·03 gm. daily for 5 days). Recently, however, a case has come under our control which developed marked cyanosis, accompanied by the presence of abnormal pigments in the blood-stream, on the usual doses of atebrin and plasmochin.

Below we outline the history and laboratory examination of the case.

A female aged 24 years was seen by us in the laboratory and stated that her first attack of malaria occurred on August 17th, 1936. Before that date she had never had malaria. For the attack she was treated with 2 × 0·5 gm. intramuscular injections of quinine bihydrochloride plus 3 × 1·0 gm. injections. After the quinine injections she was given 2 × 0·01 gm. injections of plasmochin simplex. This treatment was followed by a complete course of atebrin. Ten

* Health Section, League of Nations, Dorothea Simmons Research Funds.

†Barkan and Schales (1937), and later Barkan (1937), have described a pigment in blood which they have named 'pseudo-methaemoglobin.' Since, however, this term was used by Fairley and Bromfield previously (1937) to the work of Barkan, in describing a pigment having its absorption maximum at 624 $m\mu$ in blackwater fever, it would appear that the term 'pseudo-methaemoglobin' should be reserved for the pigment originally described by Fairley and Bromfield (1937) which has its absorption maximum at 622–624 $m\mu$ (Foy and Kondi, 1938).

days after the cessation of the atebrin she took 1·2 gm. oral quinine sulphate daily for 10 days, followed by 0·8 gm. per week for 2 months. She completed her anti-malaria treatment in about December, 1936. No immediate or delayed ill effects followed this treatment. During 1937 she was completely free from malaria and was working with the Near East Foundation in Macedonia.

On February 15th, 1938, she went down with another attack of malaria. This was undoubtedly a late clinical result of an infection in the autumn of 1937, since there is probably no transmission of malaria in December and January in Greece (Barber and Rice, 1935). For this attack she was given 3 × 1·0 gm. intramuscular injections of quinine bihydrochloride, followed by 15 × 0·1 gm. tablets of atebrin. After taking the atebrin she was given 15 × 0·01 gm. plasmochin simplex orally. Later she took quinine intermittently. If the patient actually did have this treatment, it would appear on the surface to be an adequate one ; yet on May 16th, before the reinfection commences in Greece (Barber and Rice, 1935), she went down with a smart attack of fever, with a temperature of 40° C. (= 104° F.). She was seen by the local village doctor, who took a thick blood film. On May 17th she came to the laboratory looking quite well and with no fever. A thick blood film was taken the same day, and this, together with the film taken on the previous day, contained numerous *P. vivax* (= 20–50 parasites per thick film oil immersion field).

She entered hospital on May 17th, and commenced treatment with oral atebrin and plasmochin (0·3 gm. atebrin plus 0·03 plasmochin) daily. She had a spleen I, soft but not tender, and the liver was slightly enlarged and tender.

On May 20th she was observed to be cyanotic round the lips and fingernails. The plasmochin was stopped, and a blood examination was made, as follows :—

May 20th, 1938	R.B.C.	= 3,670,000
	Haemoglobin	= Newcomer = 10·5 gm. per cent.
	C.I.*	= 0·8
	Haematocrit*	= 35·0 per cent.
Blood	M.C.V.†	= 90 c.μ
	Pijper‡	= 7·7μ–7·9μ
	Reticulocytes	= 2·0 per cent.
	Bilirubin	Direct = Negative Indirect = 1·2 mg. per cent.
Urine	Albumen	= Negative
	Urobilin	= + trace
	Bile Salts	= Negative
	Bile Acid	= Negative
	Plasmochin (chlorinil)	= ++

* = Colour index.

† = Mean corpuscular volume.

‡ = Modal mean of approximately 1,000,000 cells, taking yellow ring as diameter.

The serum, after centrifuging, was examined spectroscopically. The only pigments present were bilirubin and traces of oxyhaemoglobin (probably traumatic). There was no trace whatever of any absorption band above 600 $m\mu$.

One c.cm. of packed red cells from the centrifuged blood was lysed with 9 c.cm. of distilled water and centrifuged. The supernatant fluid, which was deep red in colour, was examined spectroscopically and found to contain a prominent band in the red above 600 $m\mu$. Examination of this band on the Hartridge reversion spectroscope showed that it occupied a position similar to that of methaemoglobin (produced by adding potassium ferricyanide to a solution of haemoglobin). A spectrogram (Plate VI) showed that the band occupied a position of 630 $m\mu$, and was therefore definitely methaemoglobin and not pseudo-methaemoglobin, as occurs always in blackwater fever. Further, the addition of 0·5 c.cm. of 10 per cent. ammonium sulphide to the supernatant fluid caused the band in the red to disappear—a characteristic of methaemoglobin but not of pseudo-methaemoglobin. For comparison, in Plate VI the spectrogram of the pigment in the red from the case of plasmochin poisoning is taken on the same plate with a specimen of pseudo-methaemoglobin from a case of blackwater fever.

On the following day, May 21st, the patient was still cyanotic, and the lysed cells still liberated methaemoglobin.

On May 22nd the patient appeared much less cyanotic, but the absorption band was still present in the red. On May 23rd the blood showed no trace of abnormal pigments. The patient made an uneventful and complete recovery.

The supernatant fluid from the lysed cells described above was placed in an incubator at 37° C. for 24 hours, and was then re-examined spectroscopically. The pigment now present had an absorption band in the red at 622 $m\mu$, and was therefore presumably pseudo-methaemoglobin, similar to that found in our cases of blackwater fever. A spectrogram of this is shown in Plate VII, taken, for purposes of comparison, on the same plate with pseudo-methaemoglobin from a case of blackwater fever. Although the inherent fuzziness, which is characteristic of the absorption band of pseudo-methaemoglobin, prevents any definite statement being made, it appears from the spectrogram in Plate VII that there is no trace of the methaemoglobin that was present on the previous day (see Plate VI). Consequently, the methaemoglobin either was converted into pseudo-methaemoglobin or disappeared on incubation. Since pseudo-methaemoglobin forms *in vitro* in weak solutions of haemoglobin incubated with serum (Fairley and Bromfield, 1937) at 40° C., the probabilities are in this instance that the methaemoglobin previously present (1) was converted into pseudo-methaemoglobin, or (2) was present in such small amounts that its absorption band was overlaid by that of psuedo-methaemoglobin, the most likely explanation being (1).

SUMMARY AND COMMENT

1. Methaemoglobin, and not pseudo-methaemoglobin, has been found spectroscopically to be the pigment present in the circulating blood in cases of plasmochin toxicity.

2. This methaemoglobin is intracorpuscular, and never appears at any time in the serum. This is in sharp contrast to the pseudo-methaemoglobin that occurs in blackwater fever, which is always free in the serum.

3. Whether, in cases of blackwater fever said to be provoked by plasmochin, the methaemoglobin is present free in the serum as a consequence of the haemolysis, is at the moment impossible to say. It is not impossible that, once the methaemoglobin has been liberated from the red cells, it may be converted into pseudo-methaemoglobin.

4. The supernatant fluid from the lysed cells of the case of plasmochin toxicity incubated with serum at 37° C. formed pseudo-methaemoglobin, but this is not unusual, since weak solution of haemoglobin incubated with serum will also form pseudo-methaemoglobin.

REFERENCES

BARBER, M. A., and RICE, J. B. (1935). Malaria studies in Greece: the malaria infection rate in nature and in the laboratory of certain species of *Anopheles* of East Macedonia. *Ann. Trop. Med. & Parasitol.*, **29**, 329.

BARKAN, G. (1937). Blutfarbstoff, Eisen, Gallenfarbstoff: neure Untersuchungen über eisenhaltige Begleiter des Hämoglobins. *Klin. Woch.*, **16**, 1265.

——— and SCHALES, O. (1937). Chemischer Aufbau und physiologische Bedeutung des 'leicht abspaltbaren' Bluteisens. *Hoppe-Seyl. Ztschr. f. Phys. Chemie*, **248**, 96.

FAIRLEY, N. H. (1937). A new blood pigment: pseudo-methaemoglobin. [Correspondence.] *Nature*, **139**, 588.

——— and BROMFIELD, R. J. (1937). Pseudo-methaemoglobin in blackwater fever and its clinical significance. *Trans. Roy. Soc. Trop. Med. & Hyg.*, **31**, 139.

FOY, H., and KONDI, A. (1938). Spectrographic analysis of pigments in serum and urine of blackwater fever. *Ibid.*, **32**, 49.

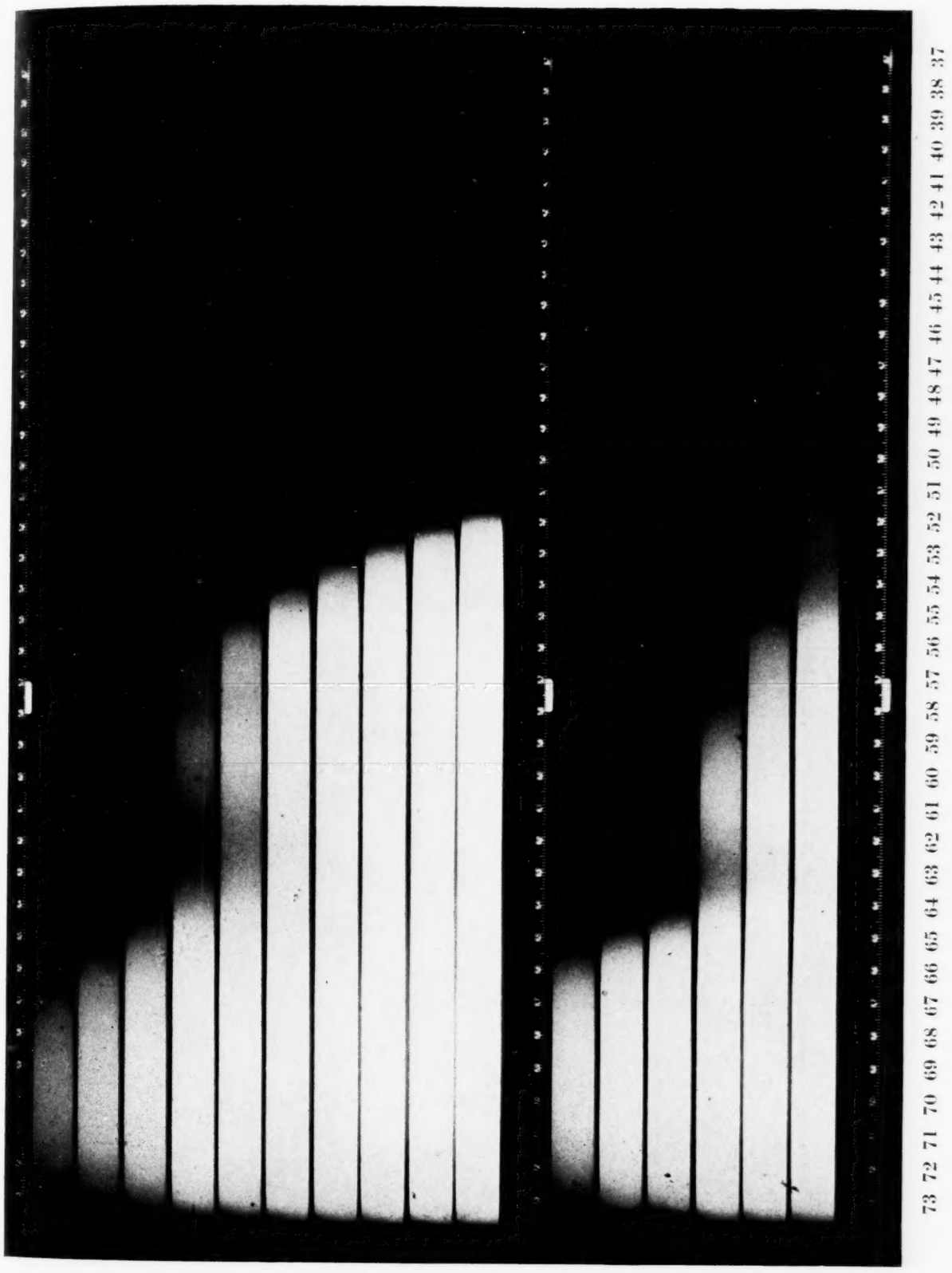
PLATE VI

EXPLANATION OF PLATE VI

Top. Pseudo-methaemoglobin from a case of blackwater fever.

Bottom. Methaemoglobin from a case of plasmochin toxicity.

PLATE VI



H. R. Grubb, Ltd., Poplar Walk, Croydon

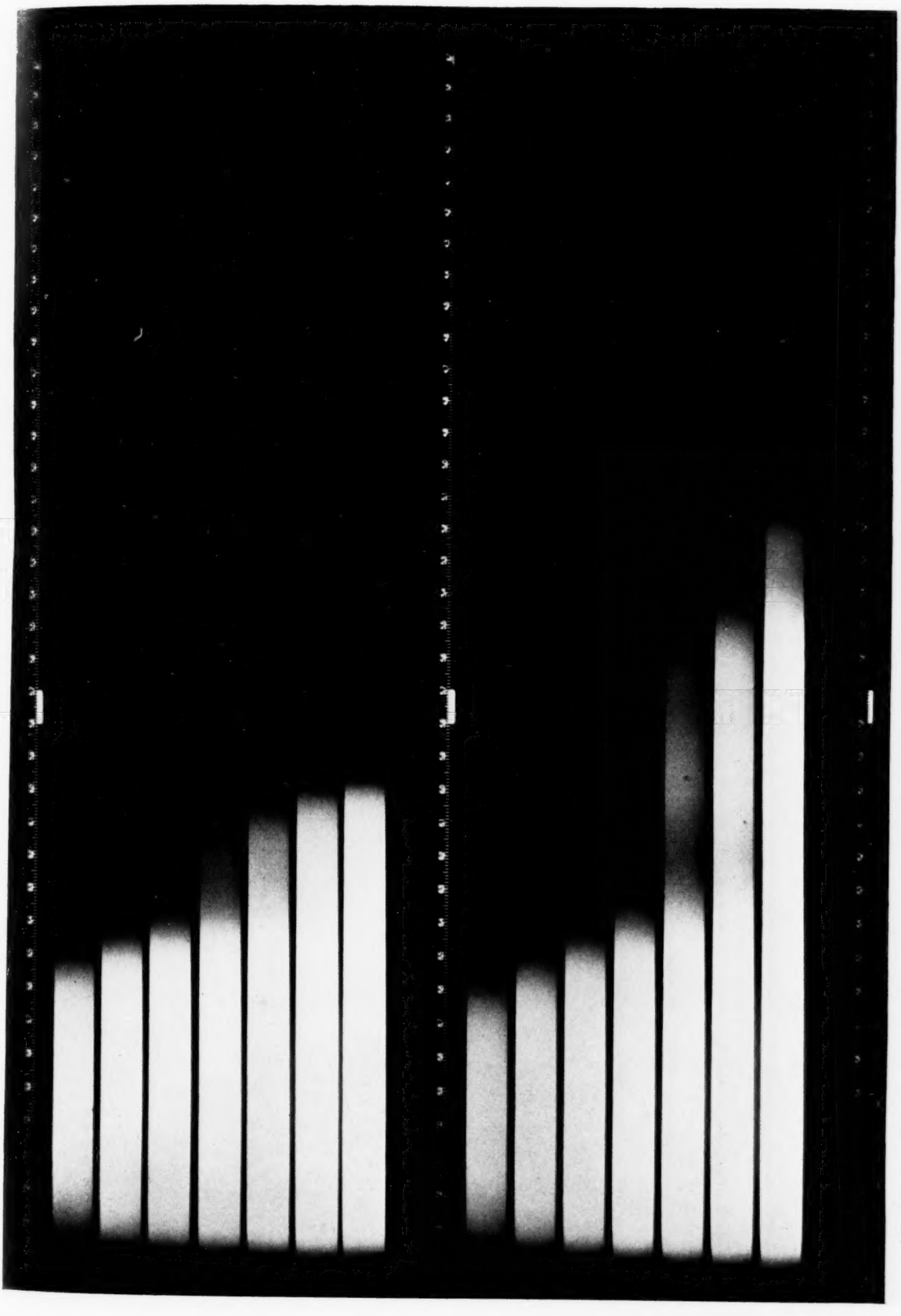
UNIV. OF MICH.

EXPLANATION OF PLATE VII

Top. Pseudo-methaemoglobin in a plasmochin case after 24 hours' incubation at 40° C.

Bottom. Pseudo-methaemoglobin from a case of blackwater fever.

PLATE VII



H. R. Grubb, Ltd., Poplar Walk, Croydon

UNIV OF

OBSERVATIONS ON THE COURSE OF *PLASMODIUM KNOWLESI* INFECTION IN MONKEYS *(MACACUS RHESUS)*, WITH NOTES ON ITS TREATMENT BY (1) ATEBRIN AND (2) 1 : 11 NORMAL UNDECANE DIAMIDINE, TOGETHER WITH A NOTE ON THE ACTION OF THE LATTER ON BIRD MALARIA

BY

SIR S. RICKARD CHRISTOPHERS*

AND

J. D. FULTON†

(*London School of Hygiene and Tropical Medicine*)

(*Received for publication August 4th*, 1938)

During experiments with *Plasmodium knowlesi* infection in monkeys (*Macacus rhesus*), extending over a period of some years and dealing with some 150 animals, considerable data have accumulated regarding the course of infection in these animals. The observations have mostly been made during ordinary routine laboratory procedure in maintaining the strain and providing material for experiments, but they have nevertheless collectively considerable interest and practical importance, especially in relation to the testing of drugs against this form of infection.

In Part I of this paper we have briefly summarized the facts regarding the course of the treated and untreated infection as seen following blood inoculation. In Part II we give some data with respect to the results of the administration of atebrin. This drug has a very powerful action against *P. knowlesi* infection in monkeys, and the data which have accumulated enable it to be used as a very convenient standard of comparison for other drugs. In Part III we give our results upon monkey and bird malaria with 1 : 11 normal undecane diamidine. This compound has been shown by King, Lourie and Yorke (1937) to have extremely powerful trypanocidal properties against both normal and arsenic-fast strains. Glyn-Hughes, Lourie and Yorke (1938) have further indicated its anti-malarial properties in human cases. The present observations were undertaken at Professor Yorke's request, to ascertain the effect of this drug against monkey and bird malaria. The special interest of the observations lies not so much in the fact that the drug has proved more effective than, or even as suitable for treatment as, the recognized anti-malarial compounds, as that it is structurally an entirely new type of compound with anti-malarial properties.

---

*Leverhulme Fellow under the Medical Research Council.

†Beit Memorial Fellow.

The strain of *P. knowlesi* used was that described by us in a previous communication (Christophers and Fulton, 1938) as having been maintained in this laboratory over a number of years by passage in monkeys. The infection in untreated animals, following a short incubation period, invariably runs a rapid course, ending in death about the 5th day from appearance of parasites in the blood. It is of interest that the fatal character of the untreated disease has not altered during six years' continuous transmission by blood inoculation (47 passages). The first invasion by parasites, which rapidly increase day by day in the blood until an infection of phenomenal intensity is reached, we have called the *primary attack*. When treatment enables an animal to survive the primary attack, it is nevertheless subject to further similar attacks, which may also prove fatal but which usually decrease in intensity, so that after a second, third or possibly fourth attack the animal will normally survive without treatment. By such time infection tends to become continuous, whilst the number of parasites in the blood becomes moderate or small. After some months the tendency to attacks, as also the number of parasites, diminishes and the animal ultimately shows more and more frequent negative blood examinations, until eventually—usually after a period of some six months or more—the blood is consistently negative and remains so even though the animals be kept under observation for long periods. The time from first appearance of parasites to the last positive examination appears to be a period of definite significance, though actually such animals are immune to reinoculation and their blood may be infective when inoculated into other monkeys over a much longer period (see observations given in Part I). The course of the disease in treated animals may thus be variously composed of the primary attack, relapse-like attacks, a state of chronic infection, and a certain total duration of infection as shown by presence of parasites in the blood, as well as by a further period in which, though clinically recovered, the animal can still be shown to be infected. All these stages, it may reasonably be supposed, may be variously influenced by the nature or dosage of particular drugs.

PART I

THE NORMAL COURSE OF *P. KNOWLESI* INFECTION

Day of first appearance of parasites. The first appearance of parasites in the blood following subcutaneous inoculation (the method used throughout in maintaining the strain) has varied from 2 to 10 days. In the majority of cases parasites first appear (about 1 in 100 microscopic fields) on the 5th and 6th day following inoculation. In 122 animals, where the time of first appearance was satisfactorily determined, the mean time was 5·6 days (see Table I).

The actual amount of blood used in inoculation has not varied greatly (0·5 to 2·0 c.cm.), but the parasite content of the blood has been very different, ranging from blood negative to microscopical examination (recovered animals)

TABLE I

Day on which parasites first appeared*	No. of animals	Mean period in days
3	6	
4	13	
5	17	
6	21	
7	32	5·6
8	9	
9	11	
10	9	
11	4	

* Counting the day of inoculation as 1.

to as much as 10,000 parasites or more per 100 microscopic fields (i.e., a million parasites per c.mm.). From Table II it will be seen that the number of parasites inoculated (subcutaneously) has had but a moderate effect on the time of first appearance of parasites in the blood. A small effect, however, not amounting to more than a day or so in the mean value, appears to be displayed in the direction of some increase in the period when poorly infective material has been used. Variation in this period appeared to depend much more on the animal or some unknown factor than upon the amount of infective material inoculated as judged by the parasite content. Except in one instance (where a second inoculation was effective), subcutaneous inoculation of material known to contain parasites has never failed to produce infection in previously uninoculated animals.

TABLE II

No. of parasites per 100 microscopic fields in material inoculated	No. of animals inoculated	Range in days of incubation period	Mean period in days in which parasites first appeared
0	6	5–9	6·7
0–100	44	2–9	6·0
100–10,000	68	2–9	5·2

Day of death in primary attack. When parasites are first seen they usually number about 1 per 100 microscopic fields or less. On the succeeding day they are commonly about ten times as numerous. In rare cases, however, a period of some days may elapse before multiplication begins. By the 5th day from first appearance of parasites in the blood these number, in the majority

of cases, a hundred or more per field, the animal then rarely surviving a further cycle of schizogony. Very rarely has any animal in our series, in the absence of treatment, survived beyond the 6th day following the first appearance of parasites in the blood. In the few cases where the time has been longer, this has usually been due to the fact, as noted above, that an occasional parasite has been found on prolonged examination a short time before regular increase in numbers began. In some very large animals (over 5 kilo.) an unusually long incubation period was followed by an unusually sluggish development. In Table III is shown the day of death following first appearance of parasites for 19 animals where the necessary information was available, the day from date of inoculation being also given. These figures, showing mean periods of 5·2 and 10·6 days respectively, are supported by data for a larger number of animals (48) killed as the fatal termination approached for the preparation of parasite substance.

TABLE III

Showing number of animals dying, or killed near fatal termination, on different days from first appearance of parasites in the blood and from date of inoculation

Days	From first appearance of parasites		From date of inoculation	
	Died naturally	Killed	Died naturally	Killed
3		12		
4	5	12		
5	5	12		2
6	2	2	2	2
7	1	2	1	8
8			3	1
9	1			12
10			3	9
11			3	4
12			3	4
13			1	2
14			2	
15				4
16			2	
Means	5·2	4·2	10·6	9·0

Periodicity. The periodicity of *P. knowlesi* has been discussed by several authors. Sinton and Mulligan (1932) showed that this followed a 24-hour cycle. Brug (1934) determined the cycle to be one of 24 hours by ascertaining the area of camera lucida drawings of series of 50 parasites taken at three or four hourly intervals. The area of such a series at 9 a.m. on one day was

17·6 square micra, and at 9 a.m. on the next day 17·2 square micra. Mulligan (1935) determined the percentage of 5 selected categories of parasites at 3-hourly intervals and concluded that the cycle was one of 24 hours. Jolly, Lavergne and Tanguy (1937) in one *M. cynomolgus* monkey examined over a period of 10 days observed, however, a half revolution of the cycle, and deduced from this that the cycle was one of 20 hours.

In our monkeys a periodicity of approximately 24 hours, with segmentation in the great majority of cases about 12 noon, has been maintained throughout. For this reason, examination in the forenoon usually shows a preponderance of large growing forms, which increase in size and show more and more numerous segmenting forms as the time of noon approaches. Early in the afternoon large forms tend to be replaced by small ring forms, and a late afternoon preparation shows mainly or exclusively ring forms. Some animals show nearly all the parasites at the same approximate stage of development. In others, especially as the infection advances, the forms are more mixed.

In order to ascertain if possible how accurately the period of the cycle was one of 24 hours, a series of percentage counts was made, as given in Table IV. These counts were carried out on three successive days at 10 a.m., 11 a.m. and 12 noon (time of approaching segmentation), and at 4 p.m., 5 p.m. and 6 p.m. (time when segmentation was largely over and ring forms were predominant).

In the afternoon counts there was no evidence in the size of the rings recorded of any definite departure from a 24-hour cycle, the numbers being almost identical on the three days. The morning counts would appear, however, to show some retardation of the cycle in this animal, in that each day there were fewer rings and more large forms, indicating that segmentation had not advanced each day quite so far. Whether this effect indicated a true alteration in the cycle, or whether it was due to differing proportion of the forms in the peripheral blood and organs, or to other causes, cannot at present be stated.

Rate of parasitic increase in primary attack. Once parasites have appeared, the usual course of infection, in the absence of treatment, is an almost step-like daily increase in the number of parasites, culminating in a fatal termination with an infection of extraordinary intensity on about the 5th day. A point of interest in relation to such increasing infection in the primary attack is the rate of multiplication of the parasites, and how far such rate is demonstrated by the number of parasites in the peripheral blood.

Brug (1934) in a *Macacus rhesus* monkey found the number of parasites per c.mm. on the 10th–15th day to be 3, 205, 3,600, 97,000, 598,000 and 73,600. Excluding the last count, where terminal conditions may have modified the numbers, this would give a daily increase in the following multiples: 68, 18, 27 and 6; or, taking the total number reached in three generations, a mean rate of multiplication of $\sqrt[3]{32,300} = 31{\cdot}8$. Christophers (1937), in typical cases dying on the 5th day, found a mean value for successive days of 1, 5, 200, 2,000 and 20,000 parasites per 100 fields.

TABLE IV

Showing total and percentage counts of parasites at 10 a.m., 11 a.m., and 12 noon, and at 4 p.m., 5 p.m., and 6 p.m. on 3 successive days (2nd to 4th from first appearance of parasites). (Monkey no. 147)

	Observations at 10 a.m., 11 a.m., and 12 noon (segmentation period)									Observations at 4 p.m., 5 p.m., and 6 p.m. (ring stage)								
Date	31.5.38			1.6.38			2.6.38			30.5.38			31.5.38			1.6.38		
Time	10	11	12	10	11	12	10	11	12	4	5	6	4	5	6	4	5	6
Parasites per 100 fields	3	5	30	15	37	27	2500	2500	2400	6	3	7	94	154	134	4600	4600	4900
Percentage of different forms*																		
Ring (and other small forms) :																		
1/8–1/6 of red cell	3	11	8	3	4	0	0	1	0	5	1	2	0	3	0	8	2	2
1/5–1/3 „ „	15	39	73	5	9	9	1	1	0	69	61	39	73	71	53	70	75	59
1/2–3/4 „ „	4	8	8	2	1	0	0	0	0	21	30	50	26	23	45	22	23	39
Total young forms	**22**	**58**	**89**	**6**	**14**	**14**	**1**	**2**	**0**	**95**	**92**	**91**	**99**	**97**	**98**	**100**	**100**	**100**
Larger forms (schizonts)																		
1 chromatin mass	14	3	1	30	21	10	47	29	29	5	8	9	1	3	2	0	0	0
2 „ masses	5	4	1	16	19	9	53	26	14	0	0	0	0	0	0	0	0	0
3–4 „ „	11	3	0	31	18	15	17	33	25	0	0	0	0	0	0	0	0	0
Over 4 „ „	48	32	9	17	28	52	2	10	32	0	0	0	0	0	0	0	0	0
Total large forms	**78**	**42**	**11**	**94**	**86**	**86**	**99**	**98**	**100**	**5**	**8**	**9**	**1**	**3**	**2**	**0**	**0**	**0**

*100 parasites counted.

In general, in the primary attack we have found, in monkeys where the infection ran a typical course, an increase day by day of the order noted by Christophers, i.e., when parasites are first seen in routine examination they number about 1 per 100 fields ; on the next day they are commonly from 5 to 50 or more per 100 fields, and then on the three succeeding days some hundreds, some thousands and finally from 10,000 to 30,000 per 100 fields. The number per c.mm. is given approximately by 100 times these figures, the field of the microscope used containing about 400 r.b.c. The largest number counted in any non-splenectomized animal was in monkeys nos. 10 and 37, parasites being respectively 35,000 and 32,000 per 100 fields, or about 3,000,000 per c.mm. Splenectomized animals not uncommonly show figures of the latter order.

In attempting to give a mean value to such data, there are, however, many difficulties due to exceptional animals, especially more rapid increase in some cases, ending in a very large count on the 4th day or exceptionally on the 3rd day, somewhat delayed rate of increase in others, especially larger animals, and often a reduction associated with great anaemia towards the fatal termination. An important consideration is also the time when the observations are made, the number of rings seen in the afternoon being often unduly disproportionate to the previous morning's count and usually some twice or more times greater than the count on the succeeding morning, when the same generation has reached a larger stage. The accompanying table (Table V) gives counts for a number of our normal and splenectomized animals, taken in routine examinations made each day before noon, using a small square aperture in the eyepiece of known area relation to the total field.

Whilst such counts are admittedly very approximate, they serve to show both the general trend of the numerical increase and the extent of variation. So far as the approximate nature of the data permit conclusions to be drawn, it would seem that (as shown also in Brug's figures) in the earlier stages of infection the factor representing the increase from one day to another is often larger than the maximum possible of merozoites, thus indicating that in the early stages parasites are being liberated which had been previously held up in the organs. This conclusion is consistent with the results of Taliaferro and Mulligan (1937, p. 90), who found the ratio of infected to uninfected red cells in the spleen and peripheral blood respectively as much as 5 : 1 in the early stages and only 1 : 1 in fatal acute cases. It is noticeable that the results with splenectomized animals are less variable, but the factor of increase is very similar to that with normal animals.

Gametocytes. Gametocytes were most commonly seen in association with the later stages of severe infections. In the case of 31 animals where the day of first appearance of these forms was noted, the majority first showed gametocytes on the 4th day after first appearance of asexual forms in the primary attack (see Table VI).

TABLE V

Showing number of parasites per 100 microscopic fields and factors of multiplication of parasites as seen in the peripheral blood for successive days after first appearance of parasites in normal and splenectomized monkeys

Monkey no.	No. of parasites per 100 microscopic fields on successive days after first appearance of parasites							Factors of multiplication for successive days					
	1	2	3	4	5	6	7	1-2	2-3	3-4	4-5	5-6	6-7
					NORMAL ANIMALS								
96	—	—	100	1,700	2,700	3,500	26,800			17	2	1	8
97	—	—	200	800	5,000	20,000				4	6	4	
98	—	6	100	2,000	10,300				17	20	5		
99	3	52	900	2,700	16,400			17	17	3	6		
100	—	20	5,900	18,900					295	3			
104	3	4	128	800	2,700	22,000		1	32	6	3	8	
109	1	60	400	4,400				60	7	11			
110	0·5	12	1,400	7,800				24	117	6			
111	2	3	200	8,300				1	66	41			
112	1	24	400	1,700	8,000	7,200		24	17	4	5	1	
113	—	36	2,200	8,000					61	4			
115	1	—	18	300	1,600	3,000	22,400			17	5	2	7
116	3	8	76	2,800	5,600	6,500		3	9	37	2	1	
117	1	8	80	2,400	800	1,600		8	10	30			
137	0·5	10	900	9,100				20	90	10			
140	2	20	62	800	5,300			10	3	13	7		
Means								**17**	**57**	**14**	**5**	**3**	**7**
					SPLENECTOMIZED ANIMALS								
77	2	500	2,500	6,600				250*	5	3			
79	0·5	8	200	3,000	24,600			16	25	15	8		
87	3	8	104	—	13,800			3	13				
88	1	52	700	9,300				52	13	13			
95	0·5	3	—	1,300	13,300			6			10		
101	—	60	900	8,000					15	9			
108	1	32	400	2,500	33,600			32	12	6	13		
122	6	14	200	3,000	31,500			2	14	15	10		
127	4	31	1,200	9,600	22,500			8	40	8	2		
126	4	40	300	1,300	14,700			10	7	4	11		
128	3	44	700	6,700				15	16	10			
132	2	10	112	3,100				5	11	28			
134	2	49	500	2,800	15,700			24	10	6	6		
136	1	29	200					29	7				
Means								**17**	**14**	**11**	**9**		

*Omitted in taking the mean.

TABLE VI

Day of appearance of gametocytes*	1	2	3	4	5	6
No. of animals		2	8	14	6	1

*Counting day of first appearance of asexual forms as 1.

Gametocytes usually numbered about 5 per 100 fields when first seen, increasing to about double this number, or in some cases (6 out of 31 animals) reaching as much as 1 or somewhat less per field. The female forms were almost invariably somewhat more numerous than the males.

Haemoglobinuria. A feature requiring some mention is the occurrence of haemoglobinuria in some animals. It is well known that *rhesus* monkeys infected with *P. knowlesi* may show haemoglobinuria accompanying the later stages of the infection. The frequency of this feature apparently varies with the strain. In the present series it has been rare, only 8 animals showing this condition out of 148 infected animals, of which at least 90 were either allowed to run to a fatal termination, or were killed in a moribund state, or had heavy infections.

Animals 39 and 40 were found with haemoglobinuria at post-mortem on the 4th and 5th day respectively of the untreated primary invasion. No. 70 developed haemoglobinuria on the 5th day of the primary attack, the condition being first noted 3 hours after the administration of atebrin. Haemoglobin continued in the urine for some 48 hours, the animal recovering with disappearance of parasites, presumably as a result of the dose of atebrin. No. 87 developed haemoglobinuria at the commencement of the second attack,when the number of parasites had not yet exceeded 2 per field. In this case, the haemoglobinuria was observed before the administration of atebrin. The condition lasted some 30 hours, the animal recovering after atebrin treatment as in the last case. No. 100 showed haemoglobinuria on the 5th day from first appearance of parasites, no drug being administered. No. 120 showed the condition on the 6th day, having received two doses of 1:11 undecane diamidine intravenously on the 3rd and 5th day. No. 144 showed haemoglobinuria at post-mortem on the 6th day, having received similar treatment. No. 128 was a splenectomized animal, haemoglobinuria being observed post mortem.

Whilst haemoglobinuria usually occurred only when the number of parasites reached a very high value, many animals with equally heavy infections failed to show it. The conclusion appears to be justified that onset of haemoglobinuria involved some condition other than degree of infection and amount of blood destruction. It would not appear that it was precipitated in these monkeys by administration of the drugs mentioned, and in two cases, nos. 70 and 87,

the animals were saved by atebrin in spite of haemoglobinuria having developed. It is of interest that the condition was seen in a recently splenectomized animal.

Total period of infection. Evidence that some animals still under observation have become spontaneously free from infection has been obtained and is summarized in Table VII. This table would indicate that spontaneous disappearance of infection may occur independent of treatment at periods of a year or more after inoculation.

TABLE VII

Showing result of reinoculation, infectivity of blood and effect of splenectomy on certain animals observed over a long period

Monkey no.	Day parasites last seen	Day blood used for inoculation and result	Day animal reinoculated and result	Day spleen removed and resulting return of parasites	Condition of animal as far as indicated by evidence
1	161		379 +		Free from infection*
5	202		348 —		Infected
11	153	208 +			,,
67	103		324 —		,,
70	163	233 +			,,
77	110			778 —	Free from infection
87	71,478			578 —	,, ,,
88	470			533 +	Infected
101	206			442 +	,,
108	117	198 —			Free from infection (?)

*Died with severe infection on reinoculation.

The available evidence therefore indicates that animals have become free from infection in 198 (?), 379, 578 and 778 days, and have given evidence of infection for 208, 233, 324, 348, 442 and 533 days respectively after inoculation.

Criteria of drug effectiveness. The very standardized character of the infection in untreated animals, and the certainty and regularity with which death occurs on about the 5th day, are useful features of *P. knowlesi* infection in relation to chemotherapeutic studies, and the fact whether or not a drug is capable of saving the life of animals in the primary attack is a first criterion which can be applied regarding the efficacy of a drug.

Following upon a treated primary attack there is commonly an interval of a certain number of days in which parasites have disappeared or become greatly reduced as shown by blood examination. This interval following the primary attack has been almost invariable after atebrin, and has also been observed, though less conspicuously, following administration of 1 : 11 undecane diamidine. The power of a drug to cause disappearance of parasites following

the treated primary attack and the mean length of the aparasitic period may be regarded as a second criterion of its efficacy (see section on interval following treated primary attack, in Part II).

Only one animal in the series after treatment of the primary attack has been allowed to go through a second attack untreated, and this animal succumbed. Of four animals in which the third attack was untreated one died. Subsequent attacks are usually milder and not necessarily fatal, even if untreated. Treatment of subsequent attacks has not therefore the same critical value as to the power to save life as treatment of the primary attack.

As the course of the infection is prolonged, it becomes more and more difficult to distinguish those exacerbations which might be regarded as attacks, the number of parasites now being smaller and rather irregular, and different animals showing a good deal of variation in the course followed. It is difficult, therefore, to apply any criterion as to the effect of a drug on the general course of infection, except with results based upon a very large number of animals, and a few exceptional cases may easily be very misleading.

Whether or not administration of any particular drug or dosage shortens the total period of infection, using this term in the clinical sense that the animal has apparently become permanently negative to microscopical examination, forms a third possible criterion. Such a period in our animals has usually extended to some six months, after which repeated examinations, sometimes carried out at short intervals over months or even years, have failed to give any further positive result. Occasionally an odd parasite has been encountered in a prolonged series of negative examinations, in one case as long as a year after the last positive find. In the majority of animals it is not difficult from the records to give the number of days from date of inoculation since when the animal has been permanently negative to routine blood examination (see section on effect of treatment on total period of infection, in Part II).

Finally, a criterion indicating complete effectiveness of a drug or treatment would be not only disappearance of the parasites to blood examination, but also evidence of sterilization of the infection, as shown by blood inoculation into susceptible animals, absence of any return of infection after splenectomy, and possibly resusceptibility of the animal to reinoculation. Such a result has never been brought about by any treatment given in our series within a period where natural spontaneous recovery could be excluded.

PART II

THE COURSE OF INFECTION IN ATEBRIN-TREATED ANIMALS

A. Effect on Incidence of Mortality in Primary Attack

In Table VIII is given the effect of different doses of atebrin administered by mouth on different dates following first appearance of parasites.

TABLE VIII

Showing effect of treatment of the primary attack with atebrin

Day after first appearance of parasites on which treatment commenced	Dosage								Summary	
	Single dose 0·05 gm.		Two doses 0·05 gm.		Single dose 0·1 gm.		Total dosage over 0·1 gm.			
	Saved	Died	Saved	Died	Saved	Died	Saved	Died	Saved	Died
1*	2				5				7	
2	2	1†	1		6			1	9	2
3	3				7	1	4		14	1
4					2		1	1	4	1
5			1		2	1	3		6	1
6						1				
Total	8	1	2		22	3	8	2	40	6

*Day of first appearance of parasites.
†Complicated by intercurrent disease (pneumonia).

Out of a total of 46 animals treated, 6 died, one complicated with intercurrent disease. Only one animal (no. 73) gave the appearance of being definitely resistant to treatment. Except for special reasons, where treatment was commenced up to the second day, animals were saved (30 animals with 3 deaths, one intercurrent, one resistant (no. 73), and one very rapidly advancing case with 30 parasites per field when treatment was commenced). Even beyond this period animals were saved unless treatment commenced at a very late stage (13 animals with 3 deaths). The effect of atebrin in these animals was very remarkable, and some that were almost moribund, with a parasite count as high as 70 per field, have recovered. One such animal recovered after onset of haemoglobinuria, which is usually a terminal condition. The effectiveness of atebrin, as judged by the first criterion of saving the life of the animal in the primary attack, is therefore very striking.

From the table it would appear that a dose of 0·05 gm., even if not repeated, was effective in saving life in the primary attack. It has to be taken into account, however, that in many cases where this dose was seen to be ineffective a further dose of the drug was given, usually 0·05 gm. or 0·1 gm. Since 36 of the animals, with 4 deaths, received a total of not more than 0·1 gm. (either as a single dose or as two doses of 0·05 gm.), it is clear that this amount was, in fact, adequate to protect against death from the primary attack in the large majority of cases. The largest total amount given (column 5) in treatment of the primary attack was never greater than 0·3 gm. in all. In the case of monkey no. 73, which appeared to be actually resistant, this amount did not prevent death,

even though treatment was commenced on the second day after appearance of parasites. The remaining deaths in this column were limited to one fatal case, where the commencement of treatment was postponed to the third day. In general, therefore, the total dose required to save life in the primary attack was 0·1 gm.

B. Interval Following Treated Primary Attack

In Table IX is given the length of interval during which parasites were not found in ordinary routine examination following treatment of the primary attack.

Table IX

Showing length of aparasitic interval following treatment of the primary attack with different doses of atebrin

Dose or doses administered	Aparasitic intervals in days	Mean aparasitic interval in days
Single dose 0·05 gm.	2, 3, 5, 5, 5, 6, 8, 9, 9	5·8
Double „ „	3, 8	5·5
Single „ 0·1 gm.	2, 2, 3, 4, 5, 5, 5, 6, 6, 6, 6, 7, 7, 8, 8, 11	5·7
Above 0·1 gm.	2, 2, 4, 5, 6, 7, 7, 11, 14	6·4
	Total	5·9

Out of 36 animals treated, the average period of the aparasitic interval was 5·9 days, ranging from 2 to 14 days. More extended examination often showed parasites to be present in small numbers during what had been taken to be an aparasitic interval, but the reduction was so considerable—below 1 parasite per 100 fields—that for practical purposes parasites had been temporarily caused to disappear. Atebrin, therefore, in the doses used, not only saved the animal from death in the primary attack, but also in all the animals saved caused the disappearance of parasites for a period, the average time during which they remained absent to ordinary microscopical examination being 6 days.

C. Effect of Treatment on the Course of the Infection Relapses

The general course of infection in animals which, owing to treatment, have not died from the primary or a succeeding attack has already been indicated, viz., that after two, three or more attacks occurring at short intervals there is a period during which parasites are more or less continuously present in moderate numbers, with lessened liability to severe attacks. This period, usually extending over some months, is followed by one in which parasites gradually disappear, and still later by apparent spontaneous recovery, when no parasites are to be found on repeated examinations of the blood so long as the animal has been under

examination. This course, it is obvious, is to some extent dependent on the treatment given, and in particular the aparasitic intervals between attacks in the early part of the course appears to be the result of the effectiveness of the atebrin given in treating the attack. Thus, in monkey no. 7, treated only with quinine (7 doses of 0·13 gm.), a continuous infection resulted, lasting 21 days, without any aparasitic interval. Similarly, animals treated with 1:11 normal undecane diamidine, as described in the next section, were in some cases saved without any definite aparasitic interval.

A numerical summary of the course of infection as seen in a large series of animals is not easy, partly owing to the difficulty of defining what should be considered an 'attack'—especially in the later course of the disease—and still more to the fact that treatment was commonly given to abort a threatened attack, and it is therefore not always possible to be sure that an attack would have actually supervened, or what its character would have been, had such treatment not been given. An attempt has, however, been made to summarize our data in Table X.

In this table figures in heavy type represent attacks in which parasites were present to the extent of at least 1,000 per 100 microscopic fields. Figures in ordinary type represent attacks in which, though no treatment was given, parasites did not reach this figure, but were over 100 per 100 fields. Figures in italics represent a similar condition to that indicated by ordinary type, but in which treatment was given, thus in many cases modifying what might have been a more severe attack. In many animals, however, attacks were aborted too early to show on such a schema, and all that can be said in this case is that the animal received a dose of the drug when parasites were judged to be increasing and likely to lead to an attack. Space does not permit of such doses being indicated in detail, but a general indication of their number is given in the column showing the total amount of drug given, the dose being usually 0·05 or 0·1 gm. at a time.

The animals have been placed in three groups, thus: (1) Those in which the primary attack was allowed to develop. In this group some animals subsequently received a very limited dosage, whilst others were more frequently treated, as is shown by the column giving total amount of drug received. (2) Those in which the primary attack was early aborted, but in which subsequent attacks were not aborted. And (3) those in which both the primary and subsequent attacks were early aborted by treatment.

The data appear to show that, whether attacks are early aborted or not, the liability to relapse is much the same. Further, in both cases the number of such relapses is moderate, the largest number noted being 8 (monkey 114). In several cases (monkeys 82, 78 and 88) there has actually been no further attack beyond the primary, using the definition of attack already given. Parasites in most cases have, however, been more or less continuously present in moderate numbers over a considerable period, a fact not indicated by the table.

TABLE X

Showing course of infection in animals variously treated with atebrin

Monkey no.	Days after inoculation on which attacks occurred	Total drug in gm.	Day parasites last seen	Total period under observation	Result of splenectomy	Total duration infection (micros.)
	Group 1					
4	**9 24** 45 **168**	0·15*	174	482		174
46	**8 17** *73* **90**	0·35	101	101		
82	**6**	0·3	50, 309	661		309
93	**7** *17*	0·3	193	475	± (7)	193
87	**7 20**	0·25	478	578	— (19)	478
95	**8** *22* **35** *95 109*	1·1	241	490	± (6)	241
114	**6** *16 25 40 65 80 90 125*	1·3	170	170		
118	**9** *29 43* 89 *131*	0·75	113	113		
	Mean duration infection (microscopic)					**279**
	Group 2					
53	*6 45* **137**	0·55	144	144		
61	*8* 16 *29 40 69*	0·35	82	82		
40	*6 16* **30**	0·30	94	94		
72	*6* **12** *84*	0·55	86	86		
38	*4 15* **24 35 63 76**	0·5	143	191		143
101	*7 34 54* 110	1·4	206	442	+ (3)	206
102	*6 22 50* 97 *153*	1·2	157	339	± (10)	157
108	*6* 40	0·9	117	230	± (9)	117
	Mean duration infection (microscopic)					**156**
	Group 3					
5	*10* 17	0·0	202	297		202
11	*10 19* 32 137	0·15	153	334		153
56	*5 41 55*	0·35	149	189		149
67	*10 25 30 36*	0·6	103	324		103
68	*7 29*	0·4	115	115		
70	*10 165*	0·75	163	1,142		163
77	*6 106*	0·5	110	778	— (22)	110
78	*5*	0·3	142, 414	608		414
79	*6 15*	0·45	98	772	± (2)	98
88	*6 56*	0·3	196, 371 470	533	+ (6)	470
	Mean duration infection (microscopic)					**207**
	Mean duration infection (microscopic)		all groups			**216**

*Also received quinine.

Of the three groups, that in which all attacks have been aborted shows perhaps on the whole the least tendency to relapse, but monkey no. 114, in which all attacks but the primary attack were treated early, received the most doses of atebrin and showed the largest number of relapses. A very large number of monkeys would clearly be required to determine whether this drug showed any special effect upon the tendency to relapse.

D. Effect of Treatment on the Total Period of Infection

Eighteen animals have been kept sufficiently long under observation to enable the period required for disappearance of parasites, as shown by blood examination, to be determined. From the data given in Table X (column 7) the mean period has been 216 days. In one or two cases alternative figures have been given where, after a long negative interval with repeated negative examinations, a positive result was obtained, usually a single parasite being seen. If the lower figure in these cases be taken, the mean value works out at 170 days, or approximately six months.

The time in the three groups has been 279, 156 and 207 days, or taking the shorter periods 208, 156 and 147 days. All the groups, therefore, have shown much the same period.

In the 6th column of the table are given the results (+ or —) of splenectomy, the former sign indicating that infection appeared spontaneously after removal of the spleen, and the latter that the animal showed no evidence of revival of infection up to the number of days noted within the brackets. In cases marked ± the period before reinoculation was too short to exclude the possibility that a return of the infection might have occurred. The results show that one animal (no. 101) was still infected 442 days after inoculation and 236 days after the last positive examination. No. 88 was still infected 553 days from inoculation. Of the remaining 7 splenectomized animals, 2 appeared to be free from infection, i.e., no. 77, negative 778 days after inoculation and 668 days after the last positive find (75 negative examinations), and no. 87, negative 578 days after inoculation and 100 days after the last positive find (22 negative examinations).

Though atebrin, therefore, showed remarkable efficacy in causing disappearance of parasites from the blood and controlling the attacks, its administration under the conditions noted gave no evidence of any effect in eradicating infection. No. 101, for example, received 14 doses of 0·1 gm. spaced over 22 weeks, whilst the two negative monkeys noted received respectively in all only 0·5 and 0·25 gm. of the drug. It would seem, therefore, that the duration of infection, as shown by microscopical examination, and the total period of infection were not influenced by the drug, even in the very considerable dosage given to animals averaging only 4 kilo. in weight.

PART III

1 : 11 NORMAL UNDECANE DIAMIDINE

Effect against Monkey Malaria

From the dose known to be tolerated by man (Glyn-Hughes, Lourie and Yorke, 1938) some preliminary experiments were made with normal monkeys, and the tolerated intravenous dose in these animals was determined to be from 2·5 to 5·0 mgm. per kilo. The lower dose was found to be repeatable at daily intervals without obvious harmful effect. The larger dose appeared to be too toxic for such continued use. By mouth, double this dose was employed in treatment. It is possible that the toxicity was somewhat greater for infected than for normal animals, judging from the observations made.

In Table XI are given data regarding 11 monkeys infected with *P. knowlesi* and treated with the drug. Six of these animals died in the primary attack, in spite of treatment, one of the deaths being probably hastened by intercurrent disease (pneumonia). A 7th animal died in a second attack in spite of treatment, the primary attack having been successfully aborted. The remaining animals survived and reached the chronic stage, showing that the drug had anti-malarial properties in its power to tide over the primary attack.

Of the animals which survived, the history is very similar to that noted in monkeys treated with atebrin. Four showed a negative period, following the treated primary attack, varying from 1 to 5 days. Nos. 126 and 127 showed five attacks during the period when they were under observation (95 days). No. 133 showed a well-marked second attack following an aborted primary attack, and subsequently chronic infection only. No. 134, following treatment of the primary attack, has up to the present shown chronic infection only.

The drug clearly has an effect against *P. knowlesi* infection in that it has saved animals from death in the primary attack and has reduced the number of parasites in some cases even where the animal succumbed. Its action appears to be considerably slower than that of atebrin, and, so far as can be judged from the small number of animals observed, it is both less markedly effective and less certain in its action than the latter drug.

Following administration, the parasite may show striking changes, especially in the younger forms which exhibit marked amoeboid shapes and fragmentation. Evidence of clumping of the pigment in the larger forms may also be observed.

Toxic effects were noted in sections of the liver and kidneys, the former showing marked fatty changes and the latter casts in the tubules. A marked increase in the amount of urine secreted was observed in several of the animals.

No indication of methaemoglobin formation was found following incubation

TABLE XI

Showing effect of treatment by 1 : 11 normal undecane diamidine upon monkeys infected with *P. knowlesi*

Monkey no.	Weight in kilo.	Dose in mgm. per. kilo.	Route	Day of infection*	Parasites per 100 micr. fields	Result
120	4·7	5·0	Intravenous	3	48	
		5·0	,,	6	800	
				7	212	Died 7th day*
121	4·1	5·0	,,	3	48	
				4	2,300	Died 4th day
126	2·7	2·5	,,	1	0·4	
		2·5	,,	4	40	Survived ; also subsequent
		2·5	,,	6	300	attacks treated by drug
				7	1,000	
				8	0·5	
127	4·1	2·5	,,	2	28	Survived ; negative period
				3	2	of 6 days ; subsequent
		2·5	,,	5	1,200	attacks treated by drug
				6	6	
129	2·9	2·5	,,	3	84	
		2·5	,,	4	2,300	Died 4th day
130	2·7	5·0	,,	3	31	
				4	86	Survived ; negative period
				6	104	of 2 days ; died second
				8	0·5	attack, though treated
133	3·6	2·5	,,	1	0·5	
		2·5	,,	2	1·0	Survived without negative
				3	4·0	period ; also subsequent
				4	4·0	attacks treated with drug
				6	1·0	
125	3·8	5·0	By mouth†	1	0·5	
				2	6·0	Died overnight on 5th day
		5·0	,,	3	1,400	with 190 parasites per
				4	7,100	field
134	3·6	10·0	,,	2	9·5	
		5·0	,,	4	400	Survived, with 1-day nega-
				5	2,300	tive period without sub-
				6	124	sequent attacks or treat-
				8	0·5	ment
143	2·3	10·0	,,	2	22	
				3	176	Died on 6th day
		10·0	,,	4	4,400	
				6	11,300	
144	2·8	10·0	,,	3	6	Died with haemoglobin-
		5·0		5	14,600	uria overnight following
						last observation

* Day of first appearance of parasites counted as 1.
† Administered with tube.

of blood with 0·002 molecular concentration of the drug for one hour at 37° C. Oxygen capacity, as tested by the method described by us, using Barcroft manometers, showed no reduction from that of normal blood, and there were no changes on spectroscopic examination.

The following results were obtained with this drug in the inhibition of O_2 uptake by 1 in 10 suspension of parasite substance, those with quinine and atebrin being given for comparison. The data show that it is a very powerful inhibitor *in vitro* (see Fulton and Christophers, 1938).

Molecular conc. of drug	Coefficient of inhibition		
	Quinine	Atebrin	1 : 11 undecane diamidine
0·000001	—	—	8·0
0·00001	3·0	9·1	51·1
0·0001	16·3	61·3	70·0
0·001	46·3	—	73·8

Effect against Bird Malaria

The accompanying table summarizes the results obtained with the drug against *P. relictum* infection in canaries of approximately 20 gm. weight, the dose being calculated in milligrams per 20 gm. body weight. The toxic dose was found to be 0·2 mgm. by peritoneal route and 0·6 mgm. by mouth. Marked fatty changes in the liver were observed in birds which had received two or more doses of 0·6 mgm. by mouth.

For therapeutic effect, birds infected by blood inoculation into the pectoral muscle received a dose of the drug from 4 to 24 hours after inoculation and 4 to 5 subsequent doses daily, the results being as shown in the table (Table XII).

There appeared to be no demonstrable effect in shortening the period when parasites first appeared, even though an almost toxic dose was employed. It was also noted that the parasites commonly appeared actually during the period when the drug was being administered.

TABLE XII

Showing effect of administration of 1 : 11 normal undecane diamidine in canaries infected by blood inoculation with *P. relictum*

Date of experiment	Bird no.	Weight in gm.	Dose in mgm. per 20 gm. body weight	Route	Day of first appearance of parasites	Remarks
13.1.38	1,234	17	0·1 6	Intraperitoneal	5	
	5	17	0·1 6	,,	3	
	6	22	0·1 6	,,	5	
	7	23	0·1 6	,,	5	
	8	21	0·1 6	,,	7	
	9	22	0·1 6	,,		Died 2nd day
	40		Control		5	
	41		,,		5	
	42		,,		3	
24.1.38	1,243	19	0·5 5	By mouth	6	
	4	22	0·5 5	,,	9	
	5	23	0·5 5	,,	4	
	6		Control		5	
	7		,,		7	
31.1.38	1,251	21	0·5 5	By mouth		*Died 5th day
	2	19	0·5 3	,,	3	
	3	18	0·5 5	,,	6	
	4	25	0·5 4	,,		*Died 5th day
	5	21	0·5	,,		Killed accidentally
	6	25	0·5 4	,,		*Died 4th day
	7		Control		8	
	8		,,		8	
	9		,,		8	
	60		,,		6	
7.2.38	1,263	19	0·1 6	Intraperitoneal	5	
	4	23	0·1 6	,,		Died 6th day
	5	22	0·1 6	,,	5	
	6	19	0·1 6	,,	6	
	7	24	0·25 6	By mouth	5	
	8	23	0·25 6	,,	5	
	9	22	0·25 6	,,	5	
	70	19	0·25 6	,,	5	
	1		Control		5	
	2		,,			Died 2nd day
	3		,,		5	
	4		,,		5	

* Liver fatty and friable.

TABLE XII—*Continued*

Date of experiment	Bird no.	Weight in gm.	Dose in mgm. per 20 gm. body weight	Route	Day of first appearance of parasites	Remarks
28.2.38	1,280	24	0·1 6	Intraperitoneal	6	
	1	22	0·1 6	,,		Died 6th day
	2	20	0·1 6	,,	6	
	3	26	0·1 2	,,		Died 3rd day
	4	24	0·3 3	By mouth		Died 4th day
	5	21	0·3 5	,,	5	
	6	24	0·3 5	,,	5	
	7	25	0·3 5	,,	7	
	8		Control		5	
	9		,,		6	
21.3.38	1,308	19	0·1 5	Intraperitoneal	6	
	9	19	0·1 5	,,	6	
	10	18	0·1 5	,,	6	
	1	22	0·1 5	,,		Not infected on 8th day
	2	18	0·3 5	By mouth	8	
	3	17	0·3 5	,,	6	
	4	21	0·3 5	,,	7	
	5	21	0·3 5	,,	7	
	6		Control		7	
	7		,,		6	
	8		,,		7	
	9		,,		8	

SUMMARY

The course of infection by *P. knowlesi* in *Macacus rhesus* monkeys, as shown by observations carried out over a number of years on some 150 animals, is described.

Data are given regarding incubation period, day of death in the primary attack, periodicity, rate of multiplication, appearance of gametocytes, occurrence of haemoglobinuria, total period of infection to spontaneous recovery and other points. Certain criteria which can be used to determine the effectiveness of drugs or methods of treatment in this infection are discussed.

Data are also given regarding the action of atebrin upon the course of the infection. Whilst remarkably effective in the saving of life and control of the attacks, the amount of atebrin administered appeared to have no influence

either upon the number of relapse attacks or upon the total duration of infection.

The effectiveness of 1 : 11 undecane diamidine against *P. knowlesi* in monkeys and against *P. relictum* in canaries has been ascertained. Whilst a definite curative effect has been demonstrated in monkey malaria, the drug is rather toxic and its effect uncertain. Its action against the parasites appears to be a delayed one. It is ineffective against the strain of bird malaria used.

ACKNOWLEDGEMENT.—We are greatly indebted to Miss A. W. Clarke for the numerous painstaking counts which are recorded in our paper.

REFERENCES

BRUG, S. L. (1934). Observations on monkey malaria. *Riv. Malariol.*, **13**, sez. I, 121.

CHRISTOPHERS, SIR S. R. (1937). Observations on *P. knowlesi* infection in *Silenus rhesus*, with description of some physico-chemical properties of isolated parasite substance. *Rep. Proc. 2nd Internat. Congr. Microbiol., Lond., 1936*, p. 282.

——— and FULTON, J. D. (1938). Observations on the respiratory metabolism of malaria parasites and trypanosomes. *Ann. Trop. Med. & Parasitol.*, **32**, 43.

FULTON, J. D., and CHRISTOPHERS, SIR S. R. (1938). The inhibitive effect of drugs upon oxygen uptake by trypanosomes (*Trypanosoma rhodesiense*) and malaria parasites (*Plasmodium knowlesi*). *Ibid.*, **32**, 77.

GLYN-HUGHES, F., LOURIE, E. M., and YORKE, W. (1938). Studies in chemotherapy. XVII: The action of undecane diamidine in malaria. *Ibid.*, **32**, 103.

JOLLY, A. M. D., LAVERGNE and TANGUY (1937). Étude expérimentale du *Plasmodium knowlesi* chez le singe et chez l'homme. *Ann. Inst. Pasteur*, **58**, 297.

KING, H., LOURIE, E. M., and YORKE, W. (1937). New trypanocidal substances. *Lancet*, **233**, 1360.

MULLIGAN, H. W. (1935). Descriptions of two species of monkey *Plasmodium* isolated from *Silenus irus*. *Arch. f. Protistenk.*, **84**, 285.

SINTON, J. A., and MULLIGAN, H. W. (1932). A critical review of the literature relating to the identification of the malarial parasites recorded from monkeys of the families Cercopithecidae and Colobidae. *Rec. Malar. Surv. India*, **3**, 357.

TALIAFERRO, W. H., and MULLIGAN, H. W. (1937). The histopathology of malaria, with special reference to the function and origin of the macrophages in defence. *Indian Med. Res. Mem.*, **29**.

A NEW TREE-HOLE BREEDING *ANOPHELES* FROM THE ISLAND OF HAINAN

BY

CH'I HO*

(*From the Department of Entomology, Liverpool School of Tropical Medicine*)

(*Received for publication September 6th*, 1938)

Anopheles sintonoides sp. nov.

ADULT. A medium-sized, unornamented, dark anopheline, resembling very closely both *A. sintoni* and *A. culiciformis*.

FEMALE. *Head.* As in *A. sintoni* and *A. culiciformis*, the inter-ocular vertex is narrow and sulcus-like, the head-scales over the occiput are of the normal expanded type, and the usual white spot on the vertex is absent. What appears to be a slight difference between this species and *A. culiciformis* is the lighter frontal tuft, which consists of a number of whitish scales instead of the rather darker ones seen in *A. culiciformis*. Palpi recognizably shorter than proboscis, averaging 2·0 mm. for the former and 2·4 mm. for the latter, this relation being intermediate between that of *A. culiciformis* and *A. sintoni*. Scales on palpi rather appressed, giving the palpi a less shaggy appearance than in *A. culiciformis*. Palpi index 0·57. Antennae and proboscis showing no difference.

Pharynx. As in others of the *Anopheles*-group (Christophers, 1933, p. 100), pharyngeal bar ill-defined, without an armature of teeth; dorsal papillae 8 in number.

Thorax. Resembling *A. culiciformis* very closely in all respects, except that it is somewhat lighter in colour. The hair-like scales, which are present in *A. sintoni* and absent in *A. culiciformis* (Puri, 1929), are absent in this species. Pleurae pale grey. Propleural hair 1, spiracular 4–6, upper sternopleural 1, lower sternopleural 3, pre-alar 1, upper mesepimeral 3–4.

Wing. Unornamented; average length 3·9 mm. Scaling of wing as in *A. culiciformis*; most scales 7-striated. Anterior forked cell one and a half times the length of its petiole and twice the length of the posterior forked cell; index 2·1.

Legs. Uniformly dark; coxae pale, devoid of scales.

Abdomen. Uniformly dark, with dark hairs; devoid of scales.

MALE. In general similar to female. Palpi equal to or a little longer than proboscis, narrowly fusiform, clubbed; hairs on club short and inconspicuous. Wings with both anterior and posterior forked cells much shorter than in the

*Research Fellow of the China Foundation for the Promotion of Education and Culture.

female ; average wing length 3·2 mm. Ungues, as in *A. culiciformis*, are without the minute spur at base.

Terminalia. Fig. 1. Coxites devoid of scales. Parabasal spines 2, each rising from a distinct lobe-like prominence ; the outer one longer and stouter than the inner ; both with their tips recurved and sharply pointed. A well-developed internal spine near the apex of coxite. Harpagones fused in middle

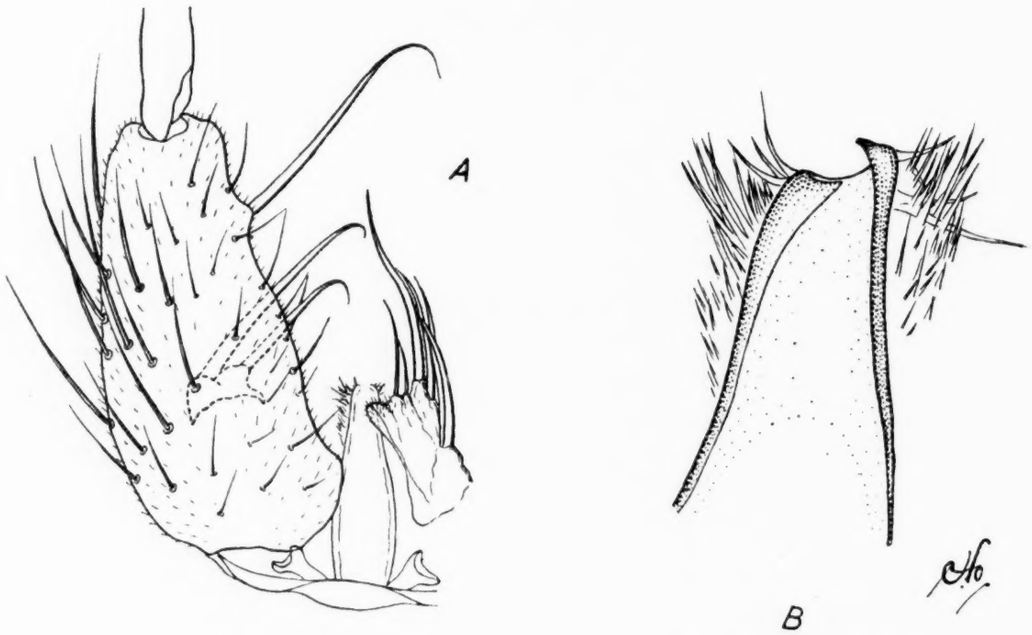


FIG. 1. *Anopheles sintonoides* sp. nov. A.—Ventral view of male terminalia ; B.—Phallosome.

line, each being three-lobed. The ventral lobe prominent, differing from *A. sintoni* in not having a small isolated hair and in the smaller size of the hairs in the group. Owing to the superimposed position of the hairs it is difficult to make out whether there are two or three in the group. The hairs on the middle and dorsal lobes are similar to those in *A. sintoni*. The fine non-papillate hairs present on the median area of the harpagones in *A. sintoni* are also present in this species, but are comparatively shorter. Phallosome large, about half the length of coxite, carrying laterally two thick clusters of spicular hairs at its distal end, the hairs which lie nearer the extremity being the longer. Ninth tergite ribbon-like, without processes.

PUPA. Fig. 2. *Paddle.* With well-marked fringe extending from near the base on external border to the base on inner border. Paddle hair straight, about twice the length of fringe hair ; accessory hair bifid.

Spine. VIII, about 15-branched, more than half the length of the segment ; accessory hair bifid, half the length of the spine or less. VII, slender, pointed, slightly bent, about one-third the length of the segment. IV–VI, straight, progressively shorter from behind forward. II–III, much shorter.

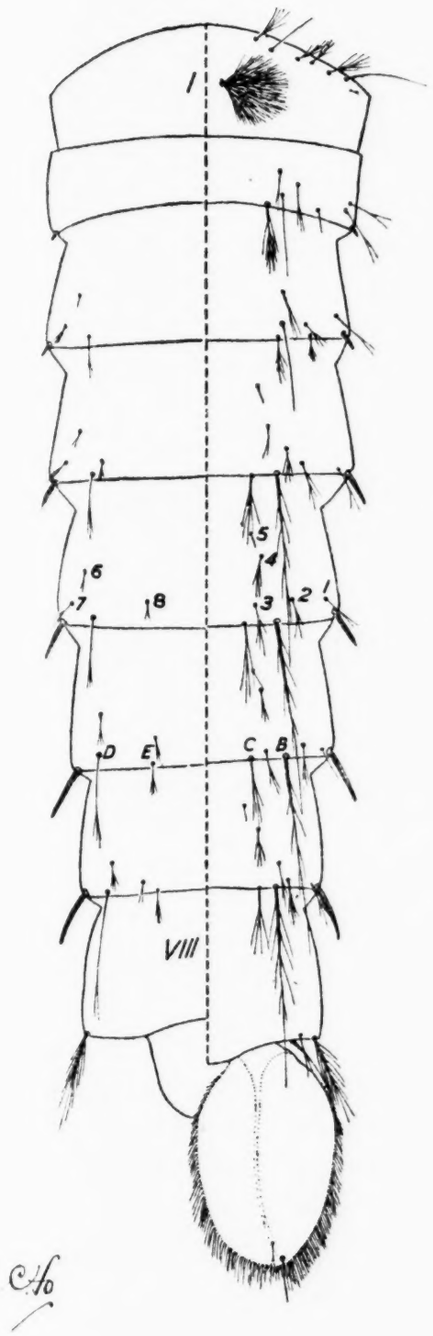


Fig. 2.

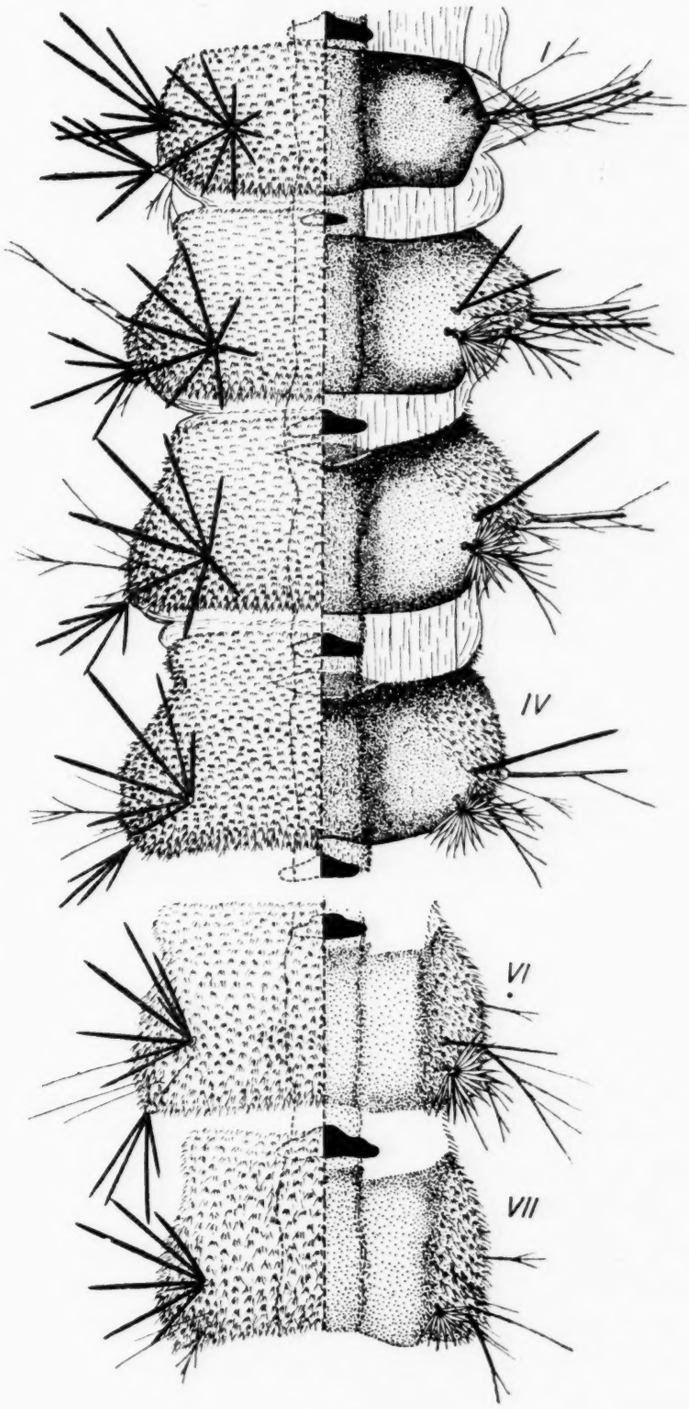


Fig. 3.

FIG. 2. *Anopheles sintonoides* sp. nov. Dorsal (right) and ventral (left) aspects of abdomen of pupa. B, C.—Large dorsal hairs; D, E.—Large ventral hairs; 1–5.—Small dorsal hairs; 6–8.—Small ventral hairs; I, VIII.—First, eighth abdominal segments.

FIG. 3. *Anopheles sintonoides* sp. nov. Dorsal (right) and ventral (left) aspects of abdomen of larva. I, IV, VI, VII.—First, fourth, sixth, seventh abdominal segments.

*Hair B.** IV–VII, considerably longer than their segments, sparsely feathered. II–III, simple, about two-thirds the length of their segments; they are not in the usual marginal positions but have moved forward a little to the discal area.

Hair C. IV–VII, half or less than half the length of their segments, split into 4–6 branches. II–III, with more branches. There is no *hair C'* on segment VI.

Hair D. VII, almost the length of the segment, delicate, bifid at the tip. IV–VI, shorter, from half to two-thirds the length of their segments, split into 3–5 branches at their tips. III, less than half the length of the segment.

Hair E. Seen in its normal position only on segments VI–VII, short, 4–branched.

Hair No. 1. Similar in its position in all the segments II–VII, bifid or trifid. There is a duplicate hair near to it on segment II.

Hair No. 2. Short, 2–5 branched, situated between and slightly anterior to the level of the spine and hair B in segments II–VII.

Hair No. 3. Short, 3–6 branched ; its position is changeable in different segments, as shown in the figure.

Hairs No. 4 and 5. Similar in their positions in all the segments III–VII; the former has 2–6 branches, the latter is simple.

Other hairs on ventral side of the abdomen as shown in figure.

LARVA.† Figs. 3 and 4. *Head.* In general, closely resembles that of *A. sintoni.* The average lengths of the clypeal hairs are : inner anterior 0·28 mm., outer anterior 0·07 mm., and posterior clypeal 0·12 mm. Frontal hairs short and feathered, with 4–8 branches. Both inner and outer sutural hairs simple. Subantennal hair differs slightly from that of *A. sintoni* in having its branches coming off nearer to the base. Antenna with a number of minute spicular projections on its inner surface. Antennal hair bifid, rising from the dorso-external surface at about one-fifth the length of the antenna from the base. Terminal hair rather stout, simple or bifid at the tip. Mentum with four black adequal and equidistant teeth on each side of the median tooth.

Thorax. The inner submedian hair on the prothorax short, with 3–5 lateral branches ; the middle hair stout, with 7–11 lateral branches and a prominent basal tubercle ; the outer hair short and simple. The innermost hair (hair no. 13) on the ventral surface of the prothorax is, like that of *A. sintoni*, stout and spinous, but differs in having 16–20 branches, instead of 4–7. The same hair on the mesothorax also is stout and spinous, having 7 or 8 branches. The hair on the neck (hair no. 14) has at the base a thickened portion from which it splits into about 10 branches, which do not divide again as in *A. sintoni.*

---

*For the terminology and general arrangement of these hairs, see Christophers, 1933, pp. 32–34.

†For reference to the hairs of the larva, see Christophers (1933) and Puri (1929).

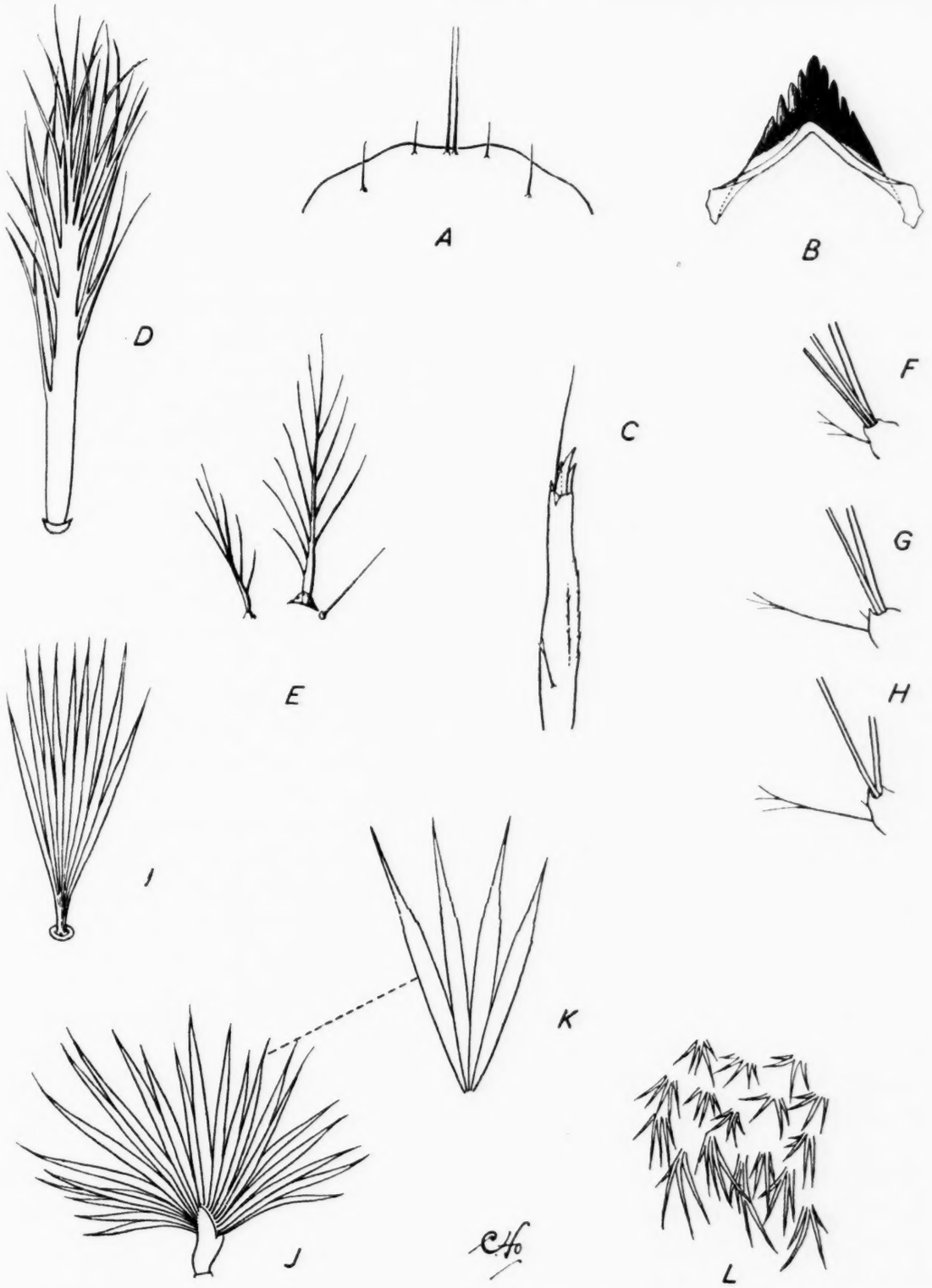


FIG. 4. *Anopheles sintonoides* sp. nov. Larval details. A.—Clypeal hairs; B.—Mentum; C.—Antenna; D.—Subantennal hair; E.—Shoulder hairs; F, G, H.—Pro-, meso-, metathoracic pleural hairs; I.—Thoracic palmate hair; J.—Palmate hair of abdominal segment IV; K.—Leaflets of the same palmate hair, more highly magnified; L.—Abdominal setae from the ventral surface of segment IV.

Hairs no. 3 and 4 on the mesothorax are simple. The metathoracic palmate hairs have about 10 lanceolate leaflets.

Pleural hairs. On the prothorax the dorsal anterior hair may be simple or finely barbed ; the short dorsal posterior hair splits into 2–4 branches, the remaining two hairs being long and simple. As in *A. sintoni*, on both meso- and metathorax the dorsal anterior hair is simple, the ventral anterior hair (the shorter of the two) finely barbed, having 3–6 scattered branches, the dorsal posterior hair simple and minute, the ventral posterior hair 2–4 branched.

Abdomen. Palmate hairs. I, minute and simple. II–VII, fully developed, with 12–20 leaflets. On IV and V, leaflets lanceolate, about 0·12 mm., filaments poorly developed, finely tapering, serration shallow, scattered along the border.

*Hair No. 2.** I, normal fine hair. II–VI, thickened and spine-like, split at the base into two branches on II, simple on III–VI. VII, normal hair.

Hair No. 5. I–II, divided near the base into 5–7 branches. III–VII, somewhat intermediate between a split and a feathered hair, having 5–7 branches.

Lateral Hairs. I–II, long, stout, feathered. III, single, half as stout as those on I and II, with short barb-like branches near the base. IV–VI, with a single long branch near the base ; in some specimens it may be shorter and 3-branched on VI. VII, short, simple.

Hair No. 9. The outermost hair on the ventral surface, stout, spine-like, split at the base into 6 branches on I and II, 4 on IV, V and VI.

Hair No. 10. Also stout and spine-like, with 6 branches on I, but normal on other segments.

Hair No. 13. This is the innermost hair on the ventral surface, and has also developed into very characteristic and stout radiate spines. It increases in size from before backwards up to segment III, after which it again diminishes. It has 10 radiate spines on I, 6–8 spines on II–VIII.

The *tergal plates* are of moderate size.

The *postspiracular hair* is short, bifid.

The *pecten* has 11–13 long and 8–10 short teeth, which more or less alternate; all have well-marked serrations at their bases. The distal end of the long teeth not split into teeth nor flattened. The pecten hair split into 5 branches near the base.

The lateral and ventral surfaces of the thorax and abdomen are densely covered with minute setae. These are very conspicuous on the ventral surface, especially towards the posterior end of each segment, and are arranged characteristically in groups of 4 or 5.

---

*This hair, which lies directly anterior and a little external to the palmate hair on II–VI, is undoubtedly hair no. 2 according to Puri's notation. But the existence at the same time of a hair close and a little posterior to the palmate hair on VI in this species leads the writer to doubt Puri's identification of hair no. 2 on that segment, since Puri regards the hair situated close and posterior to palmate hair on VI as hair no. 2 (Puri, 1929).

EGG. Unknown.

BREEDING-PLACE. The larvae were found breeding in tree-holes in the forest at an altitude of 600 m. or over. They were found in only small numbers in each tree-hole, and were twice found breeding in company with some unidentified aëdine larvae, and twice with culicine larvae.

DISTRIBUTION. Island of Hainan.

TYPES. Holotype, male, selected from a series of 7; allotype, female, selected from a series of 3; and paratypes, males and females with larvae and pupal pelts; Tsian-Fun-Lia (Mt. Pointed Peak), Kan En Hsian; and Tsi-Tse-Lia (Mt. Seven Fingers), Lin-Suai Hsian; collected and bred out by the writer, August 21st to October 20th, 1934. The holotype and allotype are deposited in the Fan Memorial Institute of Biology, Peiping, China.

The adults of *A. culiciformis*, *A. sintoni* and *A. sintonoides* sp. nov. are almost indistinguishable except for terminalic characters. The phallosome and, in the larval stage, the frontal hairs, the subantennal hair, the mentum and hair no. 13 on the prothorax, as described above, show that *A. sintonoides* is more closely related to *A. sintoni* than to *A. culiciformis*. The very characteristic stout and spinous hairs on the thorax and abdomen of the larva serve to distinguish this species from any other known *Anopheles*.

ACKNOWLEDGEMENT.—The writer would like to express here his great indebtedness to the late Dr. A. M. Evans for her guidance and for her willing assistance in many ways.

REFERENCES

CHRISTOPHERS, S. R. (1933). The fauna of British India. Diptera, vol. 4: Family Culicidae, tribe Anophelini. London.

——— and KHAZAN CHAND (1916). A tree-hole breeding *Anopheles* from southern India: *A.* (*Coelodiazesis*) *culiciformis*, Cogill. *Ind. Jl. Med. Res.*, **3**, 638.

PURI, I. M. (1929). A new tree-hole breeding *Anopheles* from south India—*Anopheles sintoni* sp. nov.—and a revised description of the larva of *A. culiciformis* Cogill. *Ibid.*, **17**, 397.

——— (1931). Larvae of anopheline mosquitoes, with full description of those of the Indian species. *Ind. Med. Res. Mem.*, **21**.

THE SIGNIFICANCE OF THE FEMALE TERMINALIA OF HOUSE-FLIES AS A GROUPING CHARACTER

BY

CH'I HO*

(From the Department of Entomology, Liverpool School of Tropical Medicine)

(Received for publication September 9th, 1938)

CONTENTS

		PAGE
I.	INTRODUCTION	287
II.	MATERIAL AND METHODS	288
III.	TYPES OF REPRODUCTION	289
IV.	A GENERAL ACCOUNT OF THE MORPHOLOGY OF THE FEMALE TERMINALIA	290
V.	A CLASSIFICATION BASED ON THE FEMALE TERMINALIA ...	297
VI.	A COMPARISON BETWEEN PATTON'S GROUPING AND THAT OF THE PRESENT WRITER	307
VII.	A COMPARISON OF DIFFERENT SYSTEMS OF CLASSIFICATION	310
VIII.	CONCLUSIONS	311
IX.	REFERENCES	311

I. INTRODUCTION

During recent years much attention has been given to the male terminalia of insects, in view of their importance in taxonomy. The female terminalia, on the other hand, have been greatly neglected, on account of a general belief that they do not provide characters of sufficient importance for classification. However, in those groups of Diptera which have been studied carefully, the female terminalia have, in some species, proved distinctive and highly specific. Thus Evans (1919) proved that the terminalia of tsetse-flies are of great value for this purpose. It is therefore obvious that this line of research still awaits investigation, and that such an investigation may lead to a better understanding of the relationships among the species.

Awati (1916, 1917) made the first comparative study of both male and female terminalia of the house-flies. As a result of his study he divided the terminalia of each sex into three distinct types. Unfortunately, he was unable to correlate his grouping of the male with that of the female, nor was the number of species available for his study very extensive. Patton (1932-37), in a series of publications, made a thorough study of the male terminalia of 41 species. His effort has resulted in a more definite knowledge of individual species, and has also made clearer the affinities and relationships among them. The present study is a continuation of Patton's work, but it approaches the problem from a different angle. Its purpose is, in the first place, to re-examine Awati's findings on female terminalia ; in the second place, to compare and correlate results found in both sexes ; and, lastly, to establish a system of natural classification for these flies based upon the terminalia.

* Research Fellow of the China Foundation for the Promotion of Education and Culture.

The flies under investigation belong to the genus *Musca* in its old and wide sense. In recent taxonomic studies, notably those of Malloch and Townsend, there has been a tendency to split the genus of the old concept into a number of genera and to use the name *Musca* in a more restricted sense. The present author, as a result of the studies described in this paper, has found it necessary to reconsider the status of the names at present assigned to these genera.

Owing to the limitation of space, the writer proposes to publish here only the general result of the study, and to leave the detailed description of the terminalic characters of individual species to a later communication.

II. MATERIAL AND METHODS

The examination of the terminalia of a female house-fly almost always necessitates the extraction of these organs from their position of repose within the abdomen. Occasionally a specimen may evert them when it is killed, but this is quite exceptional.

When the specimen is fresh the process of extraction is simple; the terminalia can be squeezed out without difficulty by compressing the soft abdomen with a pair of forceps. If, however, the specimen has been allowed to become dry with the terminalia retracted, a dissection must be resorted to. The method employed is as follows: The whole abdomen is removed from the fly and placed in a test-tube containing 10 per cent. KOH. For two reasons it has been found preferable to work with the whole abdomen rather than with the terminal portion: first, because if the fly dissected is a mature and gravid female, eggs or larvae will be recovered during the process of maceration, thus showing whether the species is oviparous or larviparous; and, secondly, because there is no danger of damaging the terminalia which are concealed within the abdomen. The length of time necessary to leave the material in KOH varies considerably with the size and condition of the specimen. As a general rule, a period of 12 hours is sufficient for most cases. The abdomen is then washed in several changes of water, its dissolved tissues are expressed, and meanwhile a search for eggs or larvae is made among the expressed tissues.

The extraction of terminalia is carried out under the binocular microscope with the aid of two blunt needles. In a few easier cases, the terminalia become displayed when pressure is applied on the fluid contents of the abdomen. But this does not generally happen, and the operation needs trained and steady hands. The anterior four abdominal segments are teased off first. The fifth segment, containing the telescoped terminalia, is placed with the ventral side uppermost. While the left-hand needle is holding the fifth segment in position, the right-hand needle gently pushes the terminalia outwards from within. In so doing, the anal segment of the terminalia may be seen protruding from the posterior end of the fifth segment. When the protruding portion is of sufficient length to lay on the needle-point, the extraction of the terminalia can be completed by placing the right-hand needle on the anal segment and gradually

pulling it away from the left-hand needle. The traction must be slow and steady, and the pressure applied by the needle-point must not be more than is necessary to pull the tip of terminalia along, otherwise the needle-point is liable to penetrate the chitinous plates of the anal segment. When fully extended, great care must be taken not to twist the organ.

For dehydration and mounting, the usual method is adopted.

The material used in this investigation was largely given by Professor W. S. Patton from his long-accumulated collection of the genus. All the specimens had been carefully determined and their corresponding males recently studied by him. The author's own collection from China furnished the remainder of the material.

The author is under a deep obligation to Professor W. S. Patton for his kindness in suggesting this interesting study and for his generosity in providing his very valuable material.

III. TYPES OF REPRODUCTION

House-flies are of peculiar interest in that they contain both larviparous and oviparous species. In the oviparous forms, some flies deposit pedicelled eggs, others non-pedicelled eggs. Three distinct types of reproduction were thus found in the insects dissected :

1. Oviparous ; depositing medium-sized, subcylindrical, non-pedicelled eggs, which are laid in batches in large numbers at a time (fig. 1, *A*).

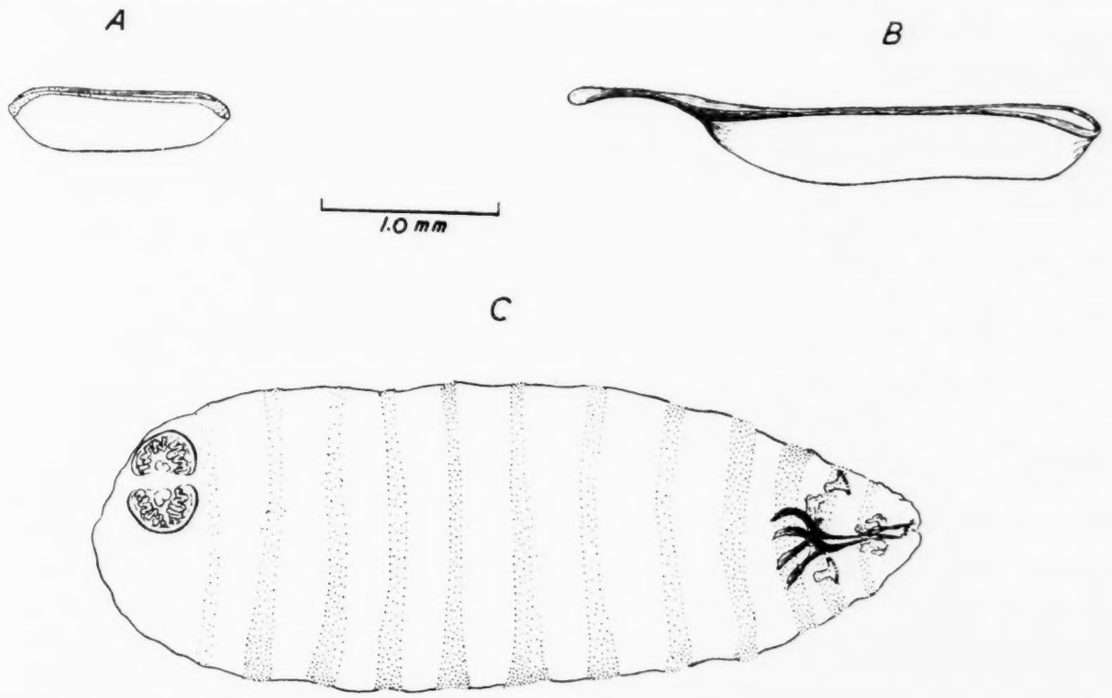


FIG. 1. Showing three different types of reproduction. *A*.—Non-pedicelled egg (*M. albina*) ; *B*.—Pedicelled egg (*E. inferior*) ; *C*.—Second stage maggot (*V. bezzii*). All dissected from gravid females.

2. Oviparous; depositing large, subcylindrical, pedicelled eggs, which are laid from about 20 to 50 at a time, singly, but close to each other, with their petioles projecting upwards (fig. 1, *B*).

3. Larviparous; depositing a single larva of the early second or the early third stage at a time (fig. 1, *C*).

This difference in the reproductive habit is a matter of primary importance, and should be borne in mind in studying the female terminalic characters. Naturally, as one would expect, a difference in function runs parallel with a difference in morphology. It will be seen in the following pages that, in accordance with the three types of reproduction, there are three markedly different types of female terminalia.

Although the existence of different reproductive habits among these higher Diptera has long been known, and three types of female terminalia were described by Awati as long ago as 1916, neither the correlation of these facts nor their important bearing upon natural classification has been properly understood.

IV. A GENERAL ACCOUNT OF THE MORPHOLOGY OF THE FEMALE TERMINALIA

The apparent number of segments which compose the abdomen of the female house-fly is nine. The first five segments*—with the exception of the first, which is usually obscured by reduction and union with the second—are comparatively unmodified and are evident without dissection. They form the visceral region of the abdomen. The segments succeeding the fifth are greatly reduced in size, and are modified to form the so-called ' tubular ovipositor', which in repose is telescoped within the fifth and is not seen without dissection. These terminal segments which are concerned with sexual mating and with the deposition of eggs or larvae are known collectively as the female terminalia.

In external morphology, the female terminalia of these flies differ from those of most other higher Diptera in the following respects :

1. Greater length.
2. Extreme reduction of the chitinous sclerites into slender rods.
3. Absence of two pairs of spiracles.

In the last point they also differ from the corresponding male flies, as the spiracles are present in the male terminalia.

It is generally agreed that the abdomen of the more primitive Diptera consists of ten segments; in muscoid Diptera, however, one of the segments in the female and two in the male have become partially or completely obliterated, there being only nine recognizable segments in the female and eight in the male. As to which two segments have disappeared in the male, three different views exist in the literature :

* Imms's (1934) designation of the abdominal segments is different from this; he writes : ' In the female *Musca* the visible segments are the third to the sixth, while the seventh to tenth segments form the retractile ovipositor.'

1. Segment VI obliterated ; segment X membranous, provided with a pair of lateral lobes (Snodgrass, 1935, pp. 605, 607).

2. Segment VIII completely disappeared ; segment X membranous, represented by anal cerci* (Awati, 1916, Table).

3. Segment VIII fused with VII ; segment X not recognizable (Townsend, 1934).

Owing to this divergence in opinion, and to the extreme difficulty in demonstrating the existing associations between the different parts, a considerable confusion in the terminology of the male terminalia has arisen. Fortunately, this is not so concerning the female terminalia. The four segments in succession are very distinct and are easily identified as segments VI, VII, VIII and IX. The missing segment is X, which has become membranous and unrecognizable.

The anal cerci on the posterior extremity of the female terminalia are considered as appendicular structures of segment X by Awati and Patton, and of segment XI by Snodgrass and Townsend.

Both fully extended and half-extended female terminalia are shown in fig. 2. The different parts are considered in greater detail below.

Length of terminalia

The terminalia vary considerably in length. The relation of the length of the terminalia to that of the body is a useful diagnostic character for primary divisions. Thus :

Non-pedicelled-egg-laying species : from 2/3 to 1 of body length.

Pedicelled-egg-laying species : from 1/2 to 2/3 of body length.

Larviparous species : about 1/3 of body length.

The next useful character is the relative length of segments VI, VII and VIII. They are more or less of equal length in larviparous species, but decrease progressively towards the extremity of the abdomen in both groups of oviparous species.

The first three segments with their conspicuous intersegmental membranes comprise the greater part of the total length of the terminalia ; the terminal segment (IX), which is short, and in front of which there is no conspicuous intersegmental membrane like the preceding ones, adds little to the total length.

Theoretically, the intersegmental membrane should be equal in length to the segment immediately behind, and the total length of the terminalia should therefore be twice the length of segments VI, VII and VIII plus the length of segment IX. Thus, by assuming VI, VII, VIII and IX to be the lengths of the respective segments, the total length of the terminalia (1) will be :

$$1 = 2\,(\mathrm{VI} + \mathrm{VII} + \mathrm{VIII}) + \mathrm{IX}.$$

---

* Patton and Awati regard these organs as real cerci, while Snodgrass and Townsend consider them as different structures. The latter two authors hold the view that real cerci are not present in the male.

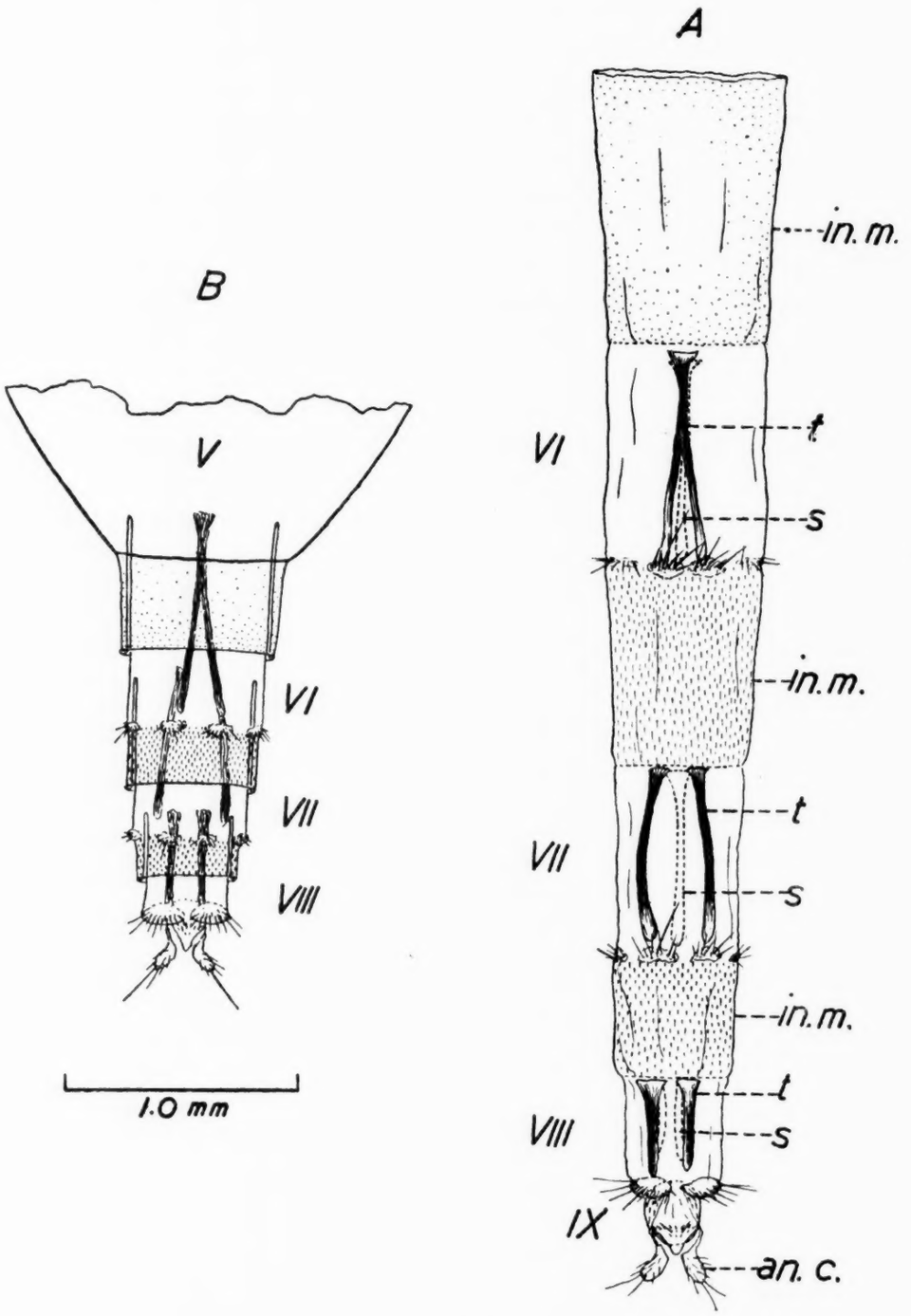


FIG. 2. General structure of female terminalia and nomenclature used. *A* and *B*.—Dorsal view of the fully and half-extended female terminalia (*M. domestica*). *V–IX*.—Segments V–IX; *in.m.*—Intersegmental membrane ; *t.*—Tergite ; *s.*—Sternite ; *an.c.*—Anal cerci.

But owing to the difficulty of mounting the terminalia in a perfectly straight and fully extended condition, the actual measurements of the total length are usually considerably lower than the value computed from the measurements of individual segments.

Awati (1918, p. 534), in giving the reason why larviparous species have shorter terminalia than oviparous species, stated that ' the larva which is hatched out from an egg in the inside of the fly has to crawl through the entire length of the ovipositor. The shorter it is the easier it is for the larva to emerge.' But his reasoning would appear to be equally applicable to the oviparous species. To the present writer, the obvious reasons for this difference are that the larvae, which are active immediately they are deposited, need no lengthy terminalia to place them into safety. Also, an increase in the width of the tube for the passage of large-sized larvae must necessarily effect its length. On the other hand, inactive eggs must be laid deeper down in places of comparative safety, and longer terminalia are therefore needed.

Intersegmental membrane

The great elongation of the terminalia is largely due to the increased development of the three intersegmental membranes between segments V and VI, VI and VII, and VII and VIII. The intersegmental membrane between segments VIII and IX is inconspicuous.

The walls of the intersegmental membranes bear innumerable minute spicules or fine hairs, which are external and forwardly directed when the terminalia are in an extended state, but become internal and backwardly directed after retraction. These spicules or fine hairs are more marked on the posterior membrane than on the anterior ones, and are more conspicuous in some species than in others.

It is the flexible intersegmental membrane that makes possible the eversion and retraction of the female terminalia, the segments themselves, with their rigid chitinous rods, remain unaltered in length but lie telescoped one within the other. It is easy to see that an intersegmental membrane must be, therefore, at least equal to the length of the segment immediately behind, in order to contain that segment in the retracted state of the terminalia.

Bristles

At the junction of the posterior ends of segments VI and VII, with the intersegmental membranes succeeding them, there are bristles either arranged in groups or individually encircling the segment. On segment VIII there are similarly situated bristles, but these are arranged differently.

The arrangement of the bristles on segment VI is practically always the same as that on VII, although the bristles on VII are often smaller in size and number. These bristles afford the most important character for differentiating different types of female terminalia. The three typical arrangements characterizing the three principal types of terminalia are shown diagrammatically in fig. 3.

But there are also other arrangements besides these. It is of special note that these bristles are greatly reduced in number in larviparous species.

The bristles on segment VIII are arranged on two pairs of tubercles situated

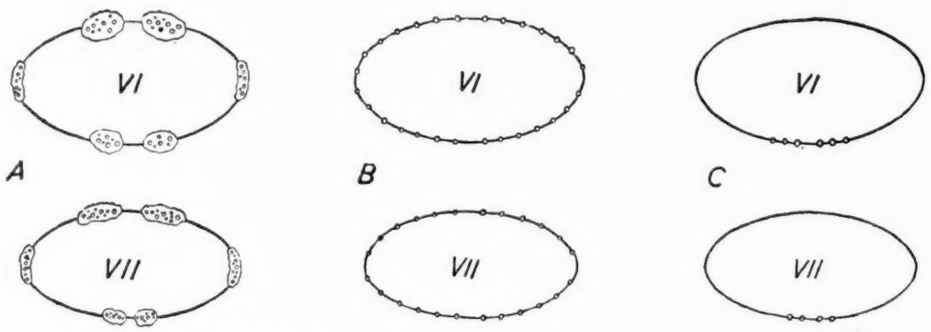


FIG. 3. Showing diagrammatically the three types of arrangement of bristles on segments VI and VII. *A.*—In six groups ; *B.*—Encircling the terminalia ; *C.*—Present only on the ventral side. *VI, VII.*—Segments VI, VII.

one on each side close to the ends of the 8th tergal sclerites and sternal sclerites. The bristles are, in most species, strong and spine-like and the tubercles are large and prominent. They may, however, be greatly reduced (fig. 5, 2). An eighth segment of this latter type is characteristic of the pedicelled-egg-laying species.

Chitinized sclerites

On segment VI the tergite is represented either by a single rod or by two separate rods, and the sternite is a single rod. Segment VII is the same as VI,

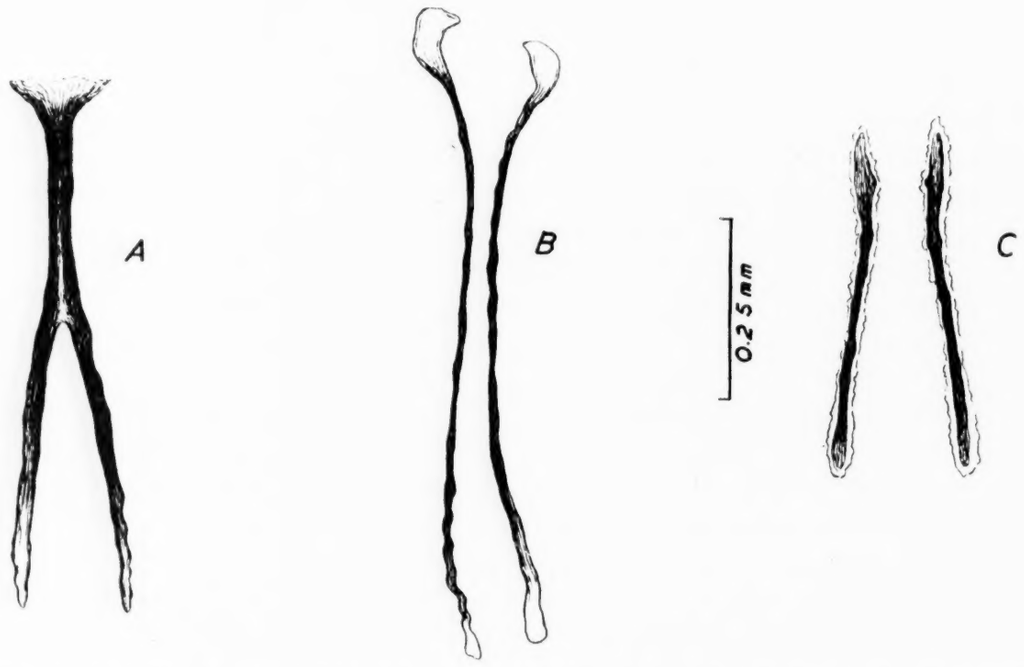


FIG. 4. Showing the three typical forms of the 6th tergite. *A.*—Forked ; *B.*—Two separate sclerites, long, close together ; *C.*—Two separate sclerites, short, wide apart.

except that the tergite is always represented by two separate rods. On segment VIII both tergite and sternite are represented by two rods.

These sclerites, representing tergites and sternites, may be well or poorly developed, and strongly or lightly chitinized. They are all rod-like, except in one species (*M. albina*), in which some of the sclerites (6th tergite, 6th and 7th sternites) assume a somewhat plate-like shape (fig. 9). The larviparous species as a rule possess poorly developed sclerites.

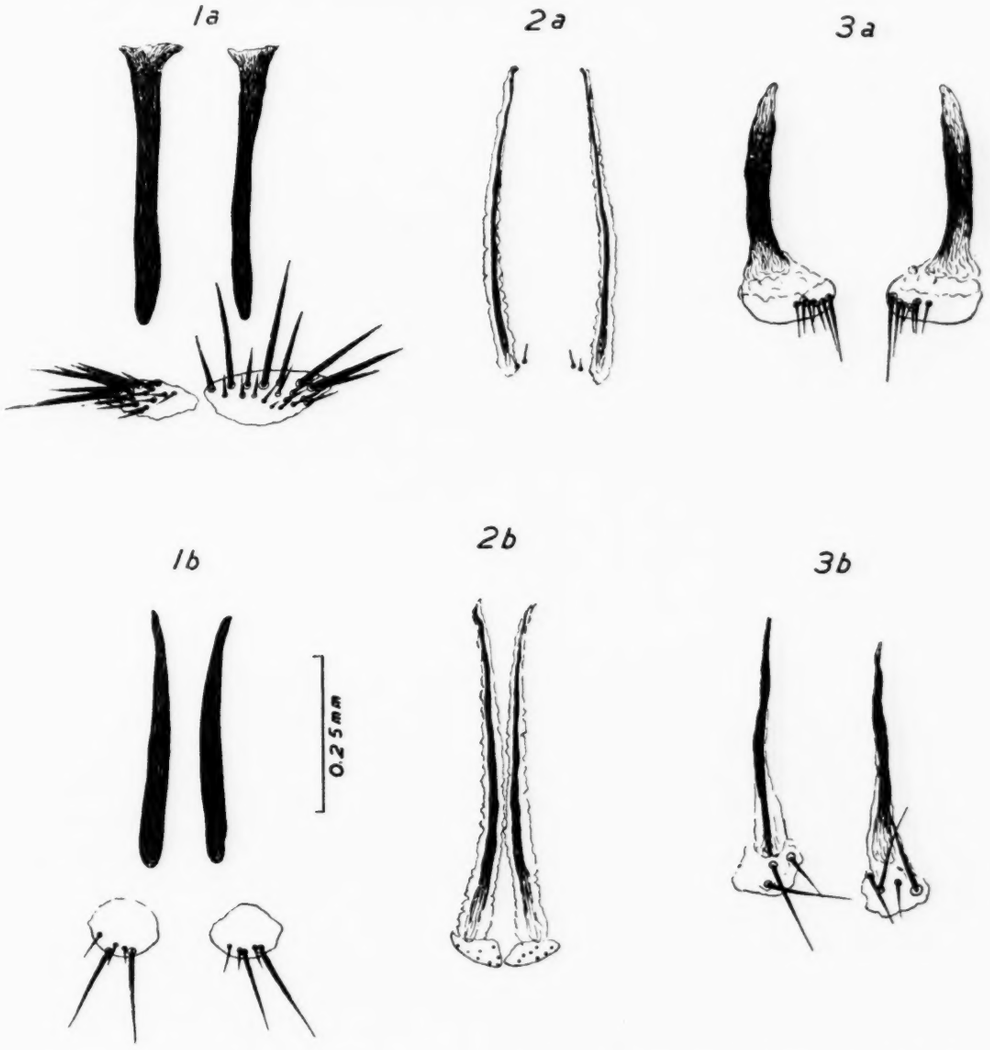


FIG. 5. Eighth tergite (1*a*, 2*a* and 3*a*) and 8th sternite (1*b*, 2*b* and 3*b*). 1.—*M. domestica*; 2.—*E. autumnalis*; 3.—*V. bezzii*.

The most important diagnostic character is to be found in the 6th tergite. It assumes a great variety of shapes, of which the three typical ones characterizing the three types of terminalia are shown in fig. 4. Other sclerites on segments VI and VII vary but little and do not afford outstanding characters of much value. On segment VIII, tergal sclerites are, in some species, longer than the

sternal sclerites, in others shorter (fig. 5). They are characters of some importance for grouping.

Genital opening

The genital opening lies on the ventral surface between segments VIII and IX, as shown in fig. 6. When at rest it is apparently closed and not easy to see. Its position is possible to determine only when an egg is being passed or when the terminalia of a pair caught *in cop.* are dissected out and cleared.

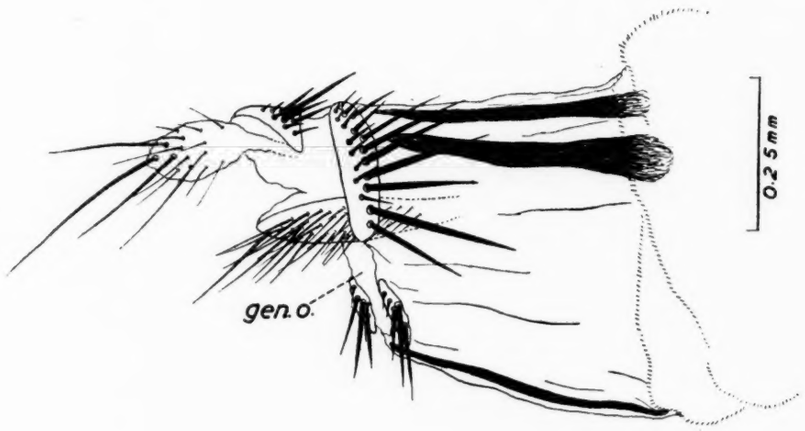


FIG. 6. Lateral view of end of female terminalia, showing the terminal segments and the genital opening (*gen.o.*).

Ninth tergal and sternal plates

The ninth tergite is a single, broad, hairy plate (fig. 7, 1*a*, 2*a*, 3*a*). It forms the upper terminal lip of the terminalia. The shape of the plate and the hairs growing on it show great variations. They are features of importance for specific identification as well as for grouping purposes.

The ninth sternite is also a single, broad, hairy plate. It forms the lower terminal lip of the terminalia. The three typical forms characterizing the three types of terminalia are shown in fig. 7. In a few cases only the plate assumes a different shape from these.

Anal cerci

Anal cerci are located on the posterior extremity of the terminalia, their basal arms lying closely applied to the posterior outer edge of the 9th tergite, one on each side. They may be pedunculate or sessile, digit-like or nearly globular, and the hairs growing on them may be short or long (fig. 6 and 7).

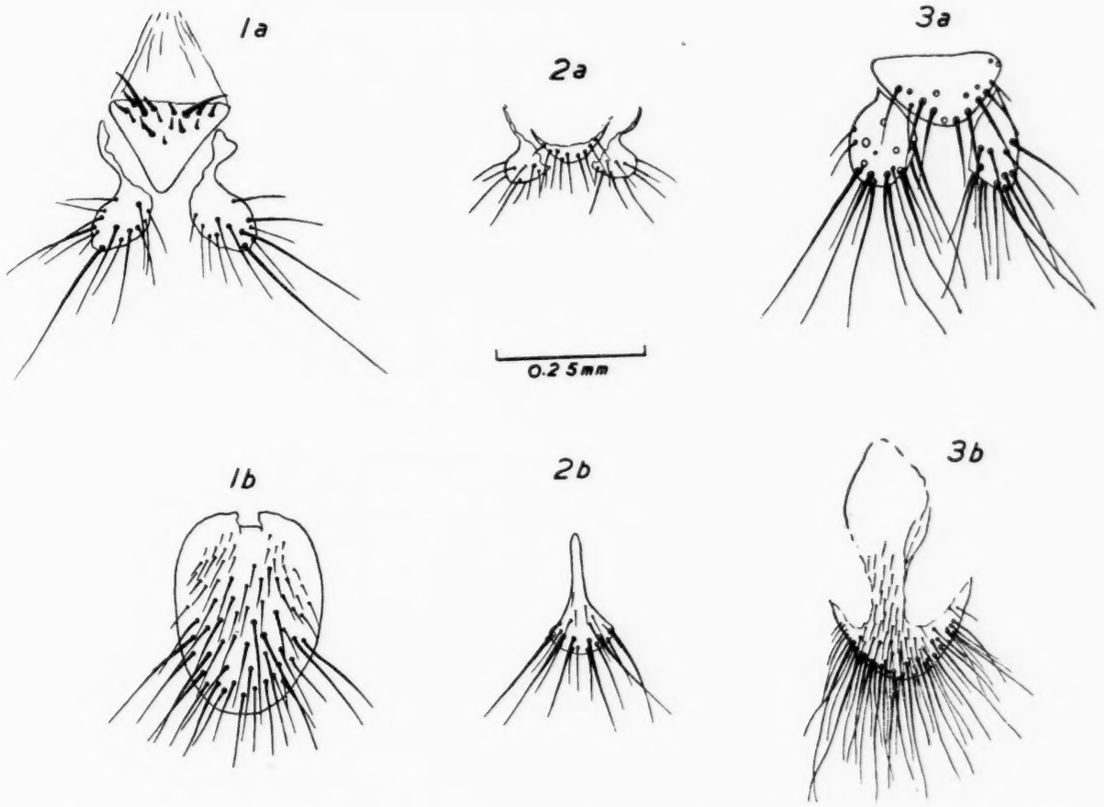


FIG. 7. Ninth tergal plate and anal cerci (1*a*, 2*a* and 3*a*) and 9th sternal plate (1*b*, 2*b* and 3*b*). 1.—*M. domestica*; 2.—*E. autumnalis*; 3.—*V. bezzii*.

V. A CLASSIFICATION BASED ON THE FEMALE TERMINALIA

The scheme of classification here adopted, as regards stem-grouping, is based entirely upon female terminalic characters, which appear to the writer, owing to their certainty and preciseness, to be the most suitable for this purpose. That the species fall into three very striking and distinct groups may easily be gathered from a study of the illustrations accompanying this work, each group being distinguished by very clear-cut characters. The grouping is strongly supported by a corresponding difference in reproductive habit, and by the male terminalic characters, which, although comparatively less distinct, follow in the main those of the female (see next section).

In the author's opinion, the characters for each of the three segregates are so distinctive and natural that he considers that each segregate should be given generic status.

The three different types of female terminalia here discussed are the same as those which have been formulated by Awati (1916).

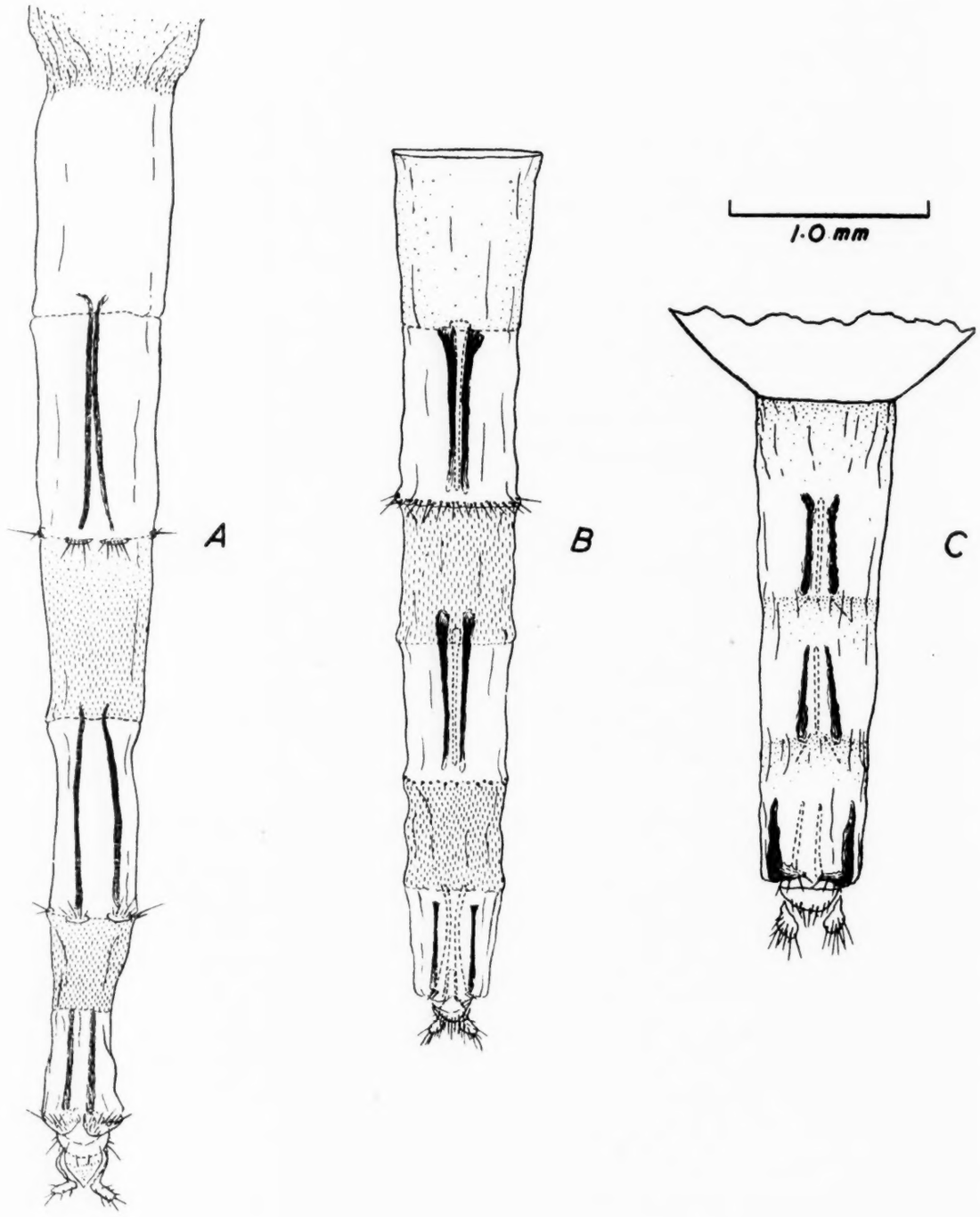


FIG. 8. Showing the general features of the three types of female terminalia. *A.—M. dasyops* ; *B.—E. inferior* ; *C.—V. convexifrons*.

Key to Genera by Female Terminalia

1. Larviparous ; terminalia short, about 1/3 of body length ; segments VI, VII and VIII more or less of equal length ; bristles on segments VI and VII reduced in number, present only on the ventral aspect of the terminalia ; 9th sternal plate anchor-shaped or scallop-shaped ... ... *Viviparomusca* T.

   Oviparous ; terminalia long, from 1/2 of body length upwards ; segments VI, VII and VIII decrease progressively in length ; bristles on segments VI and VII usually present on dorsal, ventral and lateral surfaces of terminalia ; 9th sternal plate shield-shaped or torch-shaped 2

2. Laying non-pedicelled eggs ; bristles on segments VI and VII mostly arranged in 6 groups on tubercles ; bristles on dorsal aspect of segment VIII well developed ; 8th sternite shorter than, or at most equal to, 8th sternite ; 9th sternal plate shield-shaped ... *Musca* L.

   Laying pedicelled eggs ; bristles arranged individually but uninterruptedly round the terminalia ; bristles on dorsal aspect of segment VIII reduced ; 8th sternite longer than 8th tergite ; 9th sternal plate torch-shaped ... ... *Eumusca* T.

Genus **Musca** Linnaeus

Linnaeus, 1758, Syst. Nat., X, 589.
Type species, *Musca domestica* L., 1. c. (1758).

Other names formerly accorded generic rank :
Byomya Desvoidy, 1830, Ess. Myod., 392.
Lissosterna Bezzi, 1923, Bull. Soc. Ent. Égypte, 1922, 113.
Philaematomyia Austen, 1909, Ann. & Mag. Nat. Hist., 8th ser., III, 295.
Plaxemya Desvoidy, 1830, Ess. Myod., 392.
Pristirhynchomyia Brunetti, 1910, Rec. Ind. Mus., IV, 91.

Female Terminalic Characters. Oviparous, laying non-pedicelled eggs. Length of fully extended terminalia ranging from 2/3 to about equal to that of the body ; segments VI, VII and VIII markedly and progressively decreasing in length. *Sclerites* in most species heavily chitinized and, with the exception of only one species (*M. albina*), all rod-like. The 6th tergite either Λ-shaped or separated into two parts ; in the latter case the two sclerites are often closely applied to each other. The 8th tergite longer than the 8th sternite. *Bristles* of segments VI and VII, with rare exceptions, arranged in six groups on tubercles, two dorsal, two ventral and one on either side. Bristles on segment VIII strong and spine-like, especially those on the dorsal aspect ; the four tubercles bearing these bristles large and well chitinized. *Spicules* of the posterior intersegmental membrane (membrane between VII and VIII) well developed ; those of the two anterior membranes transformed into minute hairs ; in a few species they are absent or partially so on these two membranes. In some species spicules are also present on the dorsal aspect of the membrane between VIII and IX. The *9th tergal plate* varies considerably in shape, having short or longer spines varying from a few to about ten. The *9th sternal plate* is shield-

shaped, with abundant hairs. *Anal cerci* pedunculate, semi-globose or digit-like, with longish hairs, which are longer in some species than in others.

The genus comprises the species enumerated below, the female terminalia of which have been studied by the present author :

Sixth tergite, 6th and 7th sternites plate-like (fig. 9).

M. albina Wied.

All tergites and sternites in female terminalia rod-like.

Sixth tergite Λ-shaped, the stem longer than the fork (fig. 10, *A*, *B*).

M. tempestiva Fall.
M. conducens Walk.
M. tempestatum Bezz.

Sixth tergite either Λ-shaped or separated into two sclerites ; when Λ-shaped, the stem is shorter than the fork (fig. 10, *C*, *D*, *E*).

Minute spicules absent on the distal end of segment VIII ; 9th tergal plate narrowing basally, with strong spines (fig. 11, *A*).

M. domestica L.
M. vicina Macq.
M. nebulo Fabr.
M. cuthbertsoni Patt.
M. yerburyi Patt.
M. ventrosa Wied.

Minute spicules present dorsally on the distal end of segment VIII ; 9th tergal plate broadening basally, with weak spines (fig. 11, *B*).

M. crassirostris Stein
M. dasyops Stein
M. sorbens Wied.
M. vetustissima Walk.

The genus *Musca*, which includes a wide diversity of forms, is the largest of the three genera recognized. No less than five of the genera which have been created in the past are included in it. Owing to the occurrence of transitional species it is extremely difficult to subdivide the genus.

M. albina is an isolated member of the genus and is distinguished from all the rest by having no sternopleural bristles and by the presence of plate-like sclerites in the female terminalia (fig. 9).

M. domestica, *M. vicina*, *M. nebulo*, *M. cuthbertsoni* and *M. yerburyi* form a distinct group, as has been indicated by Patton's study of the male terminalia (Patton, 1932-37). The female terminalic characters lend further proof of the correctness of this grouping. They all agree in having the 6th tergite Λ-shaped (fig. 10, *C*, *D*), and the 9th tergal plate narrowing basally and bearing some 10 strong spines (fig. 11, *A*). *M. ventrosa* appears to fall into this group, but differs in having a separated 6th tergite (fig. 10, *E*) and only about four spines on the 9th tergal plate.

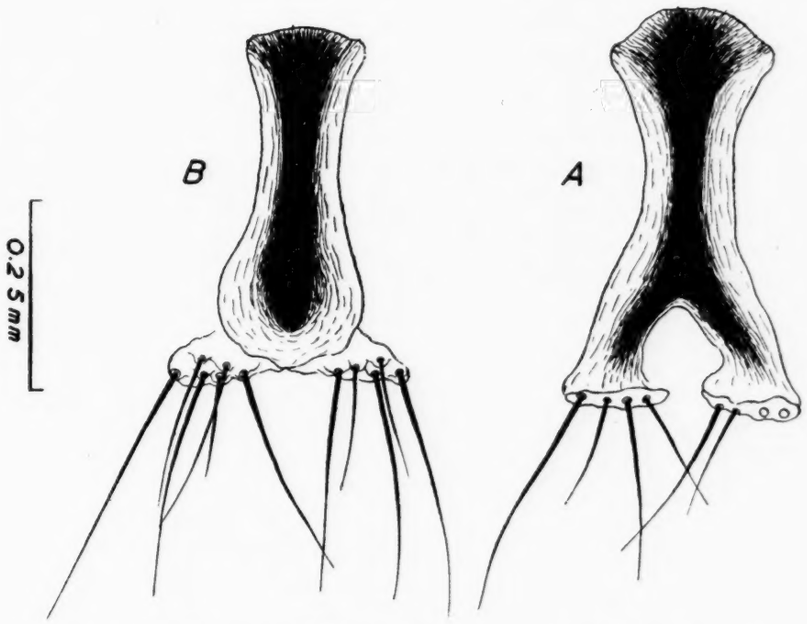


FIG. 9. Sixth tergite (*A*) and sixth sternite (*B*) of *M. albina*.

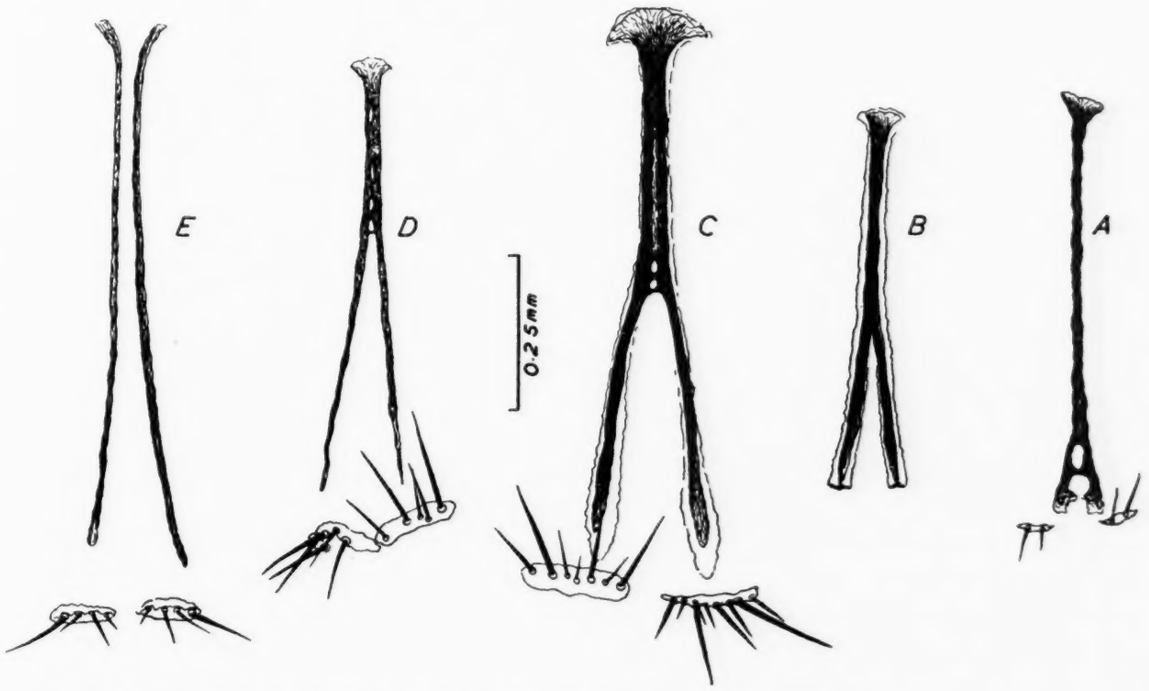


FIG. 10. Showing various forms of 6th tergite, from forking at one end to complete separation. *A.—M. tempestiva*; *B.—M. tempestatum*; *C.—M. cuthbertsoni*; *D.—M. nebulo*; *E.—M. sorbens*.

M. conducens, *M. tempestiva* and *M. tempestatum* form another group, with the last species somewhat loosely connected. All are small-sized flies, with similar male terminalia and with the female terminalia characterized by a A-shaped 6th tergite, the stem of which is longer than the fork (fig. 10, *A*, *B*).

M. crassirostris is, in several non-terminalic characters, quite a distinct species. Austen (1909) placed this species in the genus *Philaematomyia*; his classification has since been followed by most dipterologists. The female terminalic characters, however, show that *M. crassirostris* is closely related to *M. dasyops*, *M. sorbens* and *M. vetustissima*, from all of which it differs only in minor details.

M. sorbens, *M. vetustissima* and *M. dasyops* are almost indistinguishable so far as the female terminalia are concerned; but, taking non-terminalic characters into consideration, *M. sorbens* and *M. vetustissima* together form one group, and *M. dasyops* alone represents another.

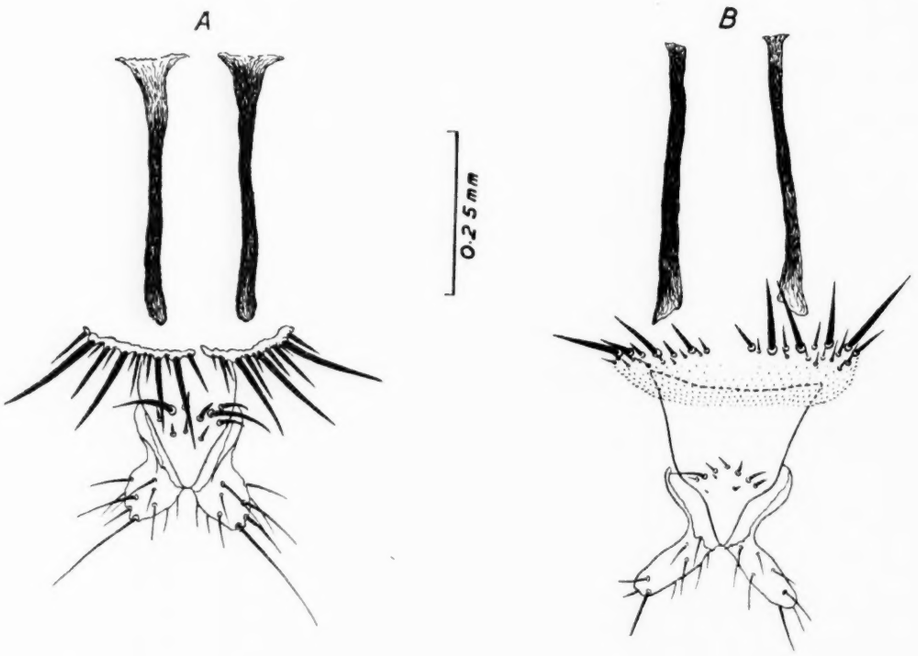


FIG. 11. Showing some distinguishing characters in segment VIII, 9th tergal plate and anal cerci. *A.—M. cuthbertsoni*; *B.—M. sorbens*.

These subdivisions, with the exception of that containing *M. sorbens* and *M. vetustissima*, have all previously been accorded generic rank. The facts brought to light by the present study lead to the conclusion that these subdivisions would be more satisfactorily designated as subgenera. The old generic names can still be used as subgeneric names, with the original concept of some of them slightly changed. It is perhaps desirable, however, that *M. sorbens* and *M. vetustissima* should have a name in keeping with the other groups, and, as there is none available, the writer proposes to name it, in honour of Professor W. S. Patton, *Pattonia* subgen. nov., with *M. sorbens* as its type species, thus:

Subgenus *Lissosterna* Bezzi
M. albina Wied.

Subgenus *Pristirhynchomyia* Brunetti
M. conducens Walk.
M. tempestiva Fall.
M. tempestatum Bezzi

Subgenus *Musca* Linnaeus
M. domestica L.
M. vicina Macq.
M. nebulo Fabr.
M. cuthbertsoni Patt.
M. yerburyi Patt.
M. ventrosa Wied.

Subgenus *Philaematomyia* Austen
M. crassirostris Stein

Subgenus *Plaxemya** Desvoidy
M. dasyops Stein

Subgenus *Pattonia* subgen. nov.
M. sorbens Wied. (type species)
M. vetustissima Walk.

Genus **Eumusca** Townsend

Townsend, 1911, Proc. Ent. Soc. Wash., XIII, 170.
Type species, *Musca autumnalis* de Geer.

Another name formerly accorded generic rank :
Ptilolepis Bezzi, 1921, Ann. Trop. Med. & Parasitol., XIV, 335.

Female Terminalic Characters. Oviparous, laying pedicelled eggs. Length of fully extended terminalia ranging from 1/2 to 2/3 that of body ; segments VI, VII and VIII markedly and progressively decreasing in length. *Sclerites* long, slender, lightly chitinized ; the two sclerites representing the 6th tergite clearly separated (fig. 12, *C*, *D*). Eighth sternite considerably longer than the 8th tergite, having a characteristic shape, as shown in fig. 12, *I*. *Bristles* on segments VI and VII arranged individually but uninterruptedly in a circle round the terminalia (fig. 12, *A*, *B*) ; those on VII much smaller in size.

* The writer has not yet had an opportunity of examining the female terminalia of *M. vitripennis*, which is the type species of the subgenus *Plaxemya*. In placing *M. dasyops* here under *Plaxemya*, he is following Malloch's classification (1925*b*), which groups together this species, *M. vitripennis* and *M. interrupta*, as belonging to his *Placomyia* (syn. of *Plexemya*).

Bristles on dorsal aspect of segment VIII either wanting or represented by two pairs which are weak and barely visible, except in *E. inferior* (compare fig. 12, *E*, ♂) ; bristles on ventral aspect of the same segment also weak but more numerous, rising from subtriangular chitinous tubercles which are close together (fig. 12, *F*). *Spicules* of the posterior intersegmental membrane well developed ; those of the anterior two membranes either wanting or transformed into sparsely distributed minute hairs. The *9th tergal plate* small, usually crescent-shaped, with not more than ten hairs (fig. 12, *H*, ♂). The *9th sternal plate* small, somewhat torch-shaped, with fewer hairs than in the

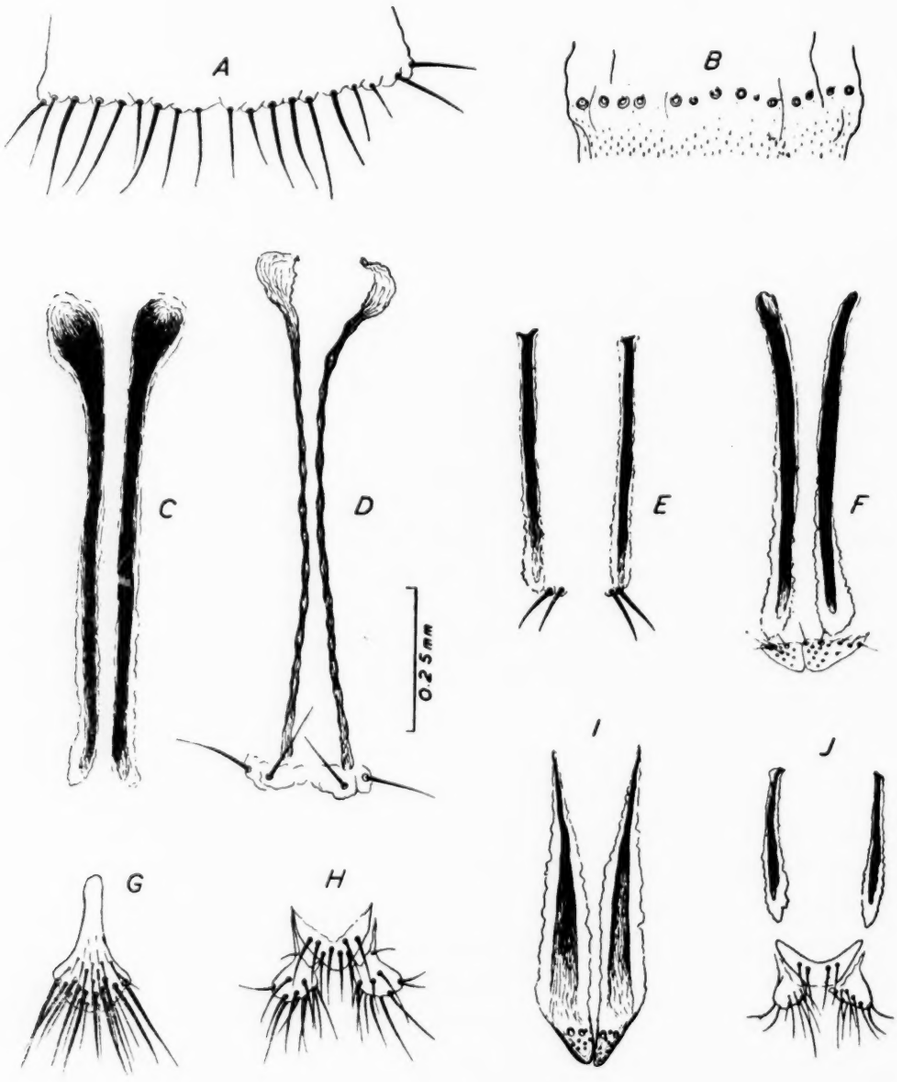


FIG. 12. Showing the chief terminalic characters of the genus and subgenera. *A*, *B*.—Bristles on segments VI and VII of *E. inferior* ; *C*.—Sixth tergite of the same ; *D*.—Sixth tergite of *E. xanthomelas* ; *E*.—Eighth tergite ; *F*.—Eighth sternite ; *G*.—Ninth sternal plate ; *H*.—Ninth tergal plate and anal cerci of *E. inferior* ; *I*.—Eighth sternite of *E. gabonensis* ; ♂.—Eighth tergite, 9th tergal plate and anal cerci of *E. craggi*.

other two genera. *Anal cerci* small, short, inconspicuously pedunculate, with short hairs (fig. 12, *H*).

The genus comprises the species enumerated below, the female terminalia of which have been studied by the present writer :

Subgenus *Eumusca* Townsend

Chitinization of sclerites weaker ; bristles on dorsum of segment VIII wanting or represented by two weak pairs (fig. 12, *D*, *I*, ♂ ; also fig. 5, 2*a*, 2*b*).

E. autumnalis de Geer
E. craggi Patt.
E. gabonensis Macq.
E. gibsoni Patt. and Cragg
**E. pattoni* Aust.
E. xanthomelas Wied.

Subgenus *Ptilolepis* Bezzi

Chitinization of sclerites stronger ; 2 pairs of well-developed bristles present on dorsum of segment VIII (fig. 12, *C*, *E*, *F*).

E. inferior Stein

This genus is remarkably homogeneous so far as the female terminalia are concerned. No subdivision is possible solely on the basis of female terminalic characters ; distinctions, while present in them, are useful only for specific identification. The erection of the genus *Ptilolepis* for *E. inferior*, although seemingly justifiable on non-terminalic characters, finds little support in female terminalic characters.

The true affinity of these flies could not have been demonstrated without an examination of the female terminalia, which differ widely from those of the other two genera. The male terminalic characters, which in some respects (i.e., in the phallosome and parameres) closely resemble those of *Viviparomusca*, are very misleading. They have caused Patton to group together all these flies and those under *Viviparomusca* into one group (Patton's *lusoria*-group). However, the distinctions indicated in the key to genera by the male terminalia (see below) will serve to separate them without difficulty.

Genus **Viviparomusca** Townsend

Townsend, 1915, Jl. Wash. Acad. Sci., V, 435.
Type species, *Musca bezzii* Patton and Cragg.

Another name formerly accorded generic rank :
Awatia Townsend, 1921, Insec. Inscit. Menst., IX, 132.

* Malloch (1925*a*, p. 135) placed *E. pattoni* under *Viviparomusca*, but Patton and Cragg's early account of the species (1913*a*, p. 17) and the female terminalia examined by the present writer both clearly prove that it should be included in *Eumusca*.

FEMALE TERMINALIC CHARACTERS. Larviparous, depositing larvae of early second or early third stage. Length of fully extended terminalia about 1/3 that of the body; segments VI, VII and VIII more or less of equal length. *Sclerites* greatly reduced, especially in the subgenus *Awatia*, those of segment VIII being better developed. The 6th tergite always represented by two widely

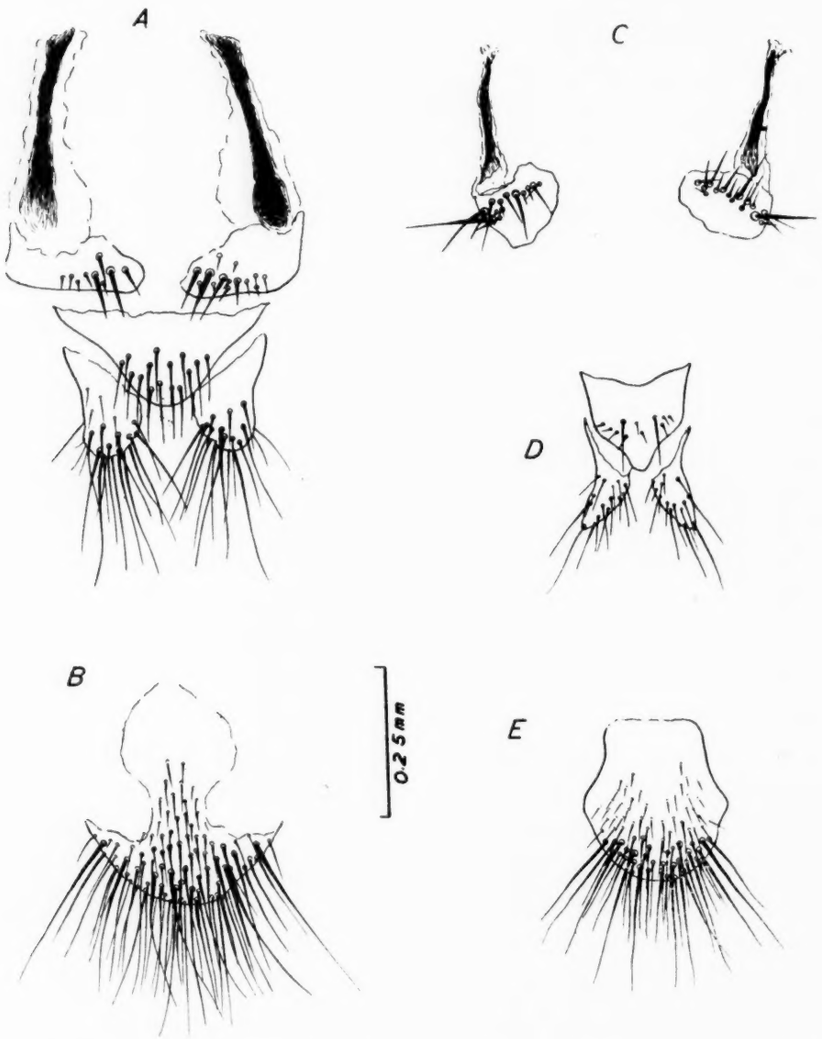


FIG. 13. Showing the distinguishing characters of the subgenera. *A.*—Eighth tergite, 9th tergal plate and anal cerci of *V. lusoria*; *B.*—Ninth sternal plate of *V. convexifrons*; *C.*—Eighth tergite of *V. planiceps*; *D.*—Ninth tergal plate and anal cerci of *V. formosana*; *E.*—Ninth sternal plate of *V. planiceps*.

separated sclerites. *Bristles* on segments VI and VII reduced, only a few present on ventral aspect, where they may be arranged individually or in two groups on tubercles. Bristles on segment VIII moderately developed, arising from four large chitinous tubercles. *Spicules* of the intersegmental membranes wanting, or partially present on the posterior one in the form of fine hairs. The 9*th*

tergal plate more or less semicircular, with 10–15 hairs (fig. 13, *A*, *D*). The *9th sternal plate* anchor-shaped in the subgenus *Viviparomusca*, scallop-shaped in the subgenus *Awatia*, with abundant long hairs. *Anal cerci* short, with abundant long hairs, sessile in subgenus *Viviparomusca*, pedunculate in subgenus *Awatia* (fig. 13, *A*, *D*).

The genus comprises the species enumerated below, the female terminalia of which have been studied by the present writer :

Subgenus *Viviparomusca*

Sclerites on segments VI and VII better developed than in *Awatia* ; 9th sternal plate anchor-shaped ; anal cerci sessile (fig. 13, *A*, *B*).

V. bezzii Patt. and Cragg
V. lusoria Wied.
V. mesopotamiensis Patt.
V. hervei Vill.
V. convexifrons Thom.

Subgenus *Awatia*

Sclerites on segments VI and VII greatly reduced ; 9th sternal plate scallop-shaped ; anal cerci pedunculate (fig. 13, *D*, *E*).

V. planiceps Wied.
V. formosana Mall.

The first five species are closely related both in structure and in habit ; they form a homogeneous group.

V. planiceps,* as has been pointed out by Patton, is a very puzzling species in regard to its true affinity to other species—a fact which is true also of *V. formosana*, a closely allied form of *planiceps*. Patton placed them in the *domestica*-group, on account of certain male terminalic characters which resembled those in *domestica*. An examination of female terminalia, however, will leave no doubt that they should be included in this genus. But they differ in a number of characters so very markedly from the other five species that they form a distinct group by themselves. The two groups are here ranked as subgenera.

VI. A COMPARISON BETWEEN PATTON'S GROUPING AND THAT OF THE PRESENT WRITER

The difference between Patton's grouping, based on male terminalic characters, and that of the present writer, based on female terminalic characters, can best be shown in the following table.

---

* Malloch placed *planiceps* and *formosana* in the subgenus *Viviparomusca* in his earlier paper (1925*b*, p. 375), but later (1929*b*, p. 111) he removed the former species to the genus *Byomya*.

TABLE I

Species	Patton's grouping of species based on male terminalia			Author's grouping of species based on female terminalia		
	domestica-group	*sorbens*-group	*lusoria*-group	Genus *Musca*	Genus *Eumusca*	Genus *Viviparomusca*
albina ...		+		+		
autumnalis ...			+		+	
bezzii ...			+			+
conducens ...		+		+		
convexifrons ...			+			+
craggi ...			+		+	
crassirostris ...		+		+		
cuthbertsoni ...	+			+		
dasyops ...			+ ?	+		
domestica ...	+			+		
formosana ...	+					+
gabonensis ...			+		+	
gibsoni ...			+		+	
hervei ...			+			+
inferior ...			+		+	
lusoria ...			+			+
mesopotamiensis			+			+
nebulo ...	+			+		
pattoni ...			+		+	
planiceps ...	+					+
sorbens ...		+		+		
tempestatum ...		+		+		
tempestiva ...		+		+		
ventrosa ...		+		+		
vetustissima ...		+		+		
vicina ...	+			+		
xanthomelas ...			+		+	
yerburyi ...	+			+		

It can easily be seen from the above table that Patton's *lusoria*-group corresponds to the present writer's *Eumusca* and *Viviparomusca* combined, while the present writer's *Musca* corresponds to Patton's *domestica*-group and *sorbens*-group combined. The only exceptions to these are *M. dasyops*, *V. planiceps* and *V. formosana*. *M. dasyops* was only provisionally placed in the *lusoria*-group in the absence of actual knowledge of its male terminalia. Its real position, as revealed by female terminalia, should be in the genus *Musca* of the present writer or, perhaps, in the *sorbens*-group of Patton's grouping. *V. planiceps* and *V. formosana*, as has been stated above, were so placed by Patton because of certain *domestica*-like male terminalic characters. Their true position in Patton's grouping should be in the *lusoria*-group and in the present writer's classification in the genus *Viviparomusca*.

On the whole, the terminalic characters of the two sexes have brought to light the same relationships among the species. The difference in grouping as shown in this table is nothing but a difference in interpretation.

That Patton's *lusoria*-group should be divided up into the two genera *Eumusca* and *Viviparomusca* of the present writer, is very evident from the female terminalia. A careful examination of Patton's illustrations of the male terminalia shows that distinguishing characters for supporting the generic divisions suggested in this paper are also present in the male terminalia. Similar evidence is available to show that the males of the species in Patton's *domestica*-group and *sorbens*-group may be grouped together in the genus *Musca* of this paper (see the following key). Patton's *domestica*-group forms a good subdivision after a stem-grouping, but does not constitute one of the stem-groups by itself.

The following key, which is prepared from Patton's descriptions and illustrations, groups the males of the species into the same three genera as are employed for the females by the present writer.

Key to Genera by Male Terminalia.*

1. Posterior process of phallosome short or moderately long, its distal end simple or slightly expanded ; anal cerci without pronounced nipple-like processes ; chitinous part of phallosome slender, usually longer than the posterior process ... ... *Musca*

   Posterior process of phallosome long, its distal end broadly expanded or Λ-forked ; anal cerci with pronounced nipple-like processes ; chitinous part of phallosome stout, usually shorter than the posterior process 2

2. Nipple-like processes of anal cerci small ; lateral processes of 5th sternite comparatively long and thin ... ... *Eumusca*

   Nipple-like processes of anal cerci large ; lateral processes of 5th sternite comparatively short and stout ... ... *Viviparomusca*

---

* For the terminology used in this key, see Patton, *Ann. Trop. Med. & Parasitol.*, **26**, p. 347, 1932.

According to this key, the following additional species, the male terminalia of which have been studied by Patton, are classified under the three genera as follows :

Genus *Musca*
M. interrupta Walk.
M. vitripennis Meig.
M. fasciata Stein
M. lusidula Loew

Genus *Eumusca*
E. munroi Patton
E. senior-whitei Patton

Genus *Viviparomusca*
V. larvipara Portsch.
V. villeneuvi Patton
V. alpesa Walk.
V. natalensis Vill.
V. greeni Patton

VII. A COMPARISON OF DIFFERENT SYSTEMS OF CLASSIFICATION

Different systems of classification by different authors are given below in tabular form (Table II) for easy reference.

TABLE II

Genera recognized by the present author	Subgenera recognized by the present author	Genera recognized by Townsend	Genera recognized by Malloch	Awati's 3 types of ♂ and ♀ terminalia		Patton's grouping of species by male terminalia
				Male	Female	
Musca	*Musca*	*Musca*	*Musca*	Type I	Type I	*domestica*-group
	Lissosterna	*Lissosterna*	*Lissosterna*			*sorbens*-group
	Pristirhynchomyia	*Pristirhynchomyia*	*Byomya*			
	Philaematomyia	*Philaematomyia*	*Philaematomyia*			
	Plaxemya	*Plaxemya*	*Placomyia*			
	Pattonia	Not recognized	Included in *Byomya*			
Eumusca	*Eumusca*	*Eumusca*	*Eumusca*	Type II	Type II	*lusoria*-group
	Ptilolepis	*Ptilolepis*	*Ptilolepis*			
Viviparomusca	*Viviparomusca*	*Viviparomusca*	*Viviparomusca*	Type III	Type III	*domestica*-group
	Awatia	*Awatia*	Included in *Byomya*			

VIII. CONCLUSIONS

1. Three types of reproduction occur in house-flies.

2. In accordance with the three types of reproduction, there are three markedly different types of female terminalia.

3. The differences in female terminalia constitute the best criteria for generic differentiation.

4. Awati's findings on female terminalia were found to be correct.

5. As a result of this study, a new system of classification is proposed.

6. A reinterpretation of Patton's grouping showed that the male terminalia indicate, although less distinctively, the same relationships among the species.

7. The different systems of classification by different authors are compared in tabular form.

IX. REFERENCES

Austen, E. E. (1909). New genera and species of blood-sucking *Muscidae* from the Ethiopian and Oriental regions, in the British Museum (Natural History). *Ann. & Mag. Nat. Hist.*, *8th ser.*, **3**, 285.

Awati, P. R. (1916). Studies in flies. II : Contributions to the study of specific differences in the genus *Musca*. 1 : Genitalia. *Ind. Jl. Med. Res.*, **3**, 510.

——— (1917). Studies in flies. III : Classification of the genus *Musca* and description of the Indian species. *Ibid.*, **5**, 160.

——— (1918). A new larviparous Philaematomyia (*Philaematomyia indica*, n.sp.). *Ibid.*, **5**, 529.

Bezzi, M. (1921). *Musca inferior*, Stein, type of a new genus of Philaematomyine flies (*Diptera*). *Ann. Trop. Med. & Parasitol.*, **14**, 333.

Brunetti, E. (1910). Revision of the Oriental blood-sucking Muscidae (*Stomoxinae, Philaematomyia*, Aust., and *Pristirhynchomyia*, gen. nov.). *Rec. Ind. Mus.*, **4**, 91.

Crampton, G. C. (1929). The terminal abdominal structures of female insects compared throughout the orders from the standpoint of phylogeny. *Jl. N.Y. Ent. Soc.*, **37**, 453.

Evans, A. M. (1919). On the genital armature of the female tsetse-flies (*Glossina*). *Ann. Trop. Med. & Parasitol.*, **13**, 31.

Hewitt, C. G. (1914). The house-fly, *Musca domestica* Linn. : its structure, habits, development, relation to disease and control. (Camb. Zool. Ser.). pp. 50-52. Cambridge.

Imms, A. D. (1934). A general textbook of entomology. 3rd ed. p. 627. London.

Lowne, B. T. (1893-95). The anatomy, physiology, morphology, and development of the blow-fly (*Calliphora erythrocephala*). vol. II, pp. 745-47. London.

Malloch, J. R. (1925*a*). Exotic *Muscaridae* (Diptera), XV. *Ann. & Mag. Nat. Hist.*, *9th ser.*, **15**, 131.

——— (1925*b*). Exotic *Muscaridae* (Diptera), XVII. *Ibid.*, *9th ser.*, **16**, 361.

——— (1928). Fauna Sumatrensis : Family *Muscidae* (Dipt.), subfamily *Muscinae* *Ent. Mitt.*, **17**, 328.

——— (1929*a*). Exotic *Muscaridae* (Diptera), XXIV. *Ann. & Mag. Nat. Hist.*, *10th ser.*, **3**, 264.

——— (1929*b*). Exotic *Muscaridae* (Diptera), XXVI. *Ibid.*, *10th ser.*, **4**, 110.

——— (1932). Exotic *Muscaridae* (Diptera), XXXVII. *Ibid.*, *10th ser.*, **10**, 297.

Patton, W. S. (1923*a*). Some Philippine species of the genus *Musca* Linnaeus. *Philipp. Jl. Sci.*, **23**, 309.

——— (1923*b*). A new Oriental species of the genus *Musca*. *Ibid.*, **23**, 323.

——— (1925). Diptera of medical and veterinary importance. I : Types of older authors in continental museums. *Ibid.*, **27**, 177.

——— (1926). The Ethiopian species of the genus *Musca* Linnaeus. *Rec. Ind. Mus.*, **28**, 29.

——— (1932). Studies on the higher Diptera of medical and veterinary importance : a revision of the species of the genus *Musca* based on a comparative study of the male terminalia. I : The natural grouping of the species and their relationship to each other. *Ann. Trop. Med. & Parasitol.*, **26**, 347.

——— (1933*a*). Studies on the higher Diptera of medical and veterinary importance : a revision of the genera of the tribe Muscini, subfamily Muscinae, based on a comparative study of the male terminalia. I : The genus *Musca* Linnaeus. *Ibid.*, **27**, 135.

PATTON, W. S. (1933*b*). Studies on the higher Diptera of medical and veterinary importance : a revision of the genus *Musca* based on a comparative study of the male terminalia. II : A practical guide to the Palaearctic species. *Ibid.*, **27**, 327, 397.

——— (1933*c*). A new haematophagous species of *Musca* from Malaya. *Ibid.*, **27**, 477.

——— (1936). Studies on the higher Diptera of medical and veterinary importance : a revision of the species of the genus *Musca* based on a comparative study of the male terminalia. III : A practical guide to the Ethiopian species. *Ibid.*, **30**, 469.

——— (1937). Studies on the higher Diptera of medical and veterinary importance : a revision of the species of the genus *Musca* based on a comparative study of the male terminalia. IV : A practical guide to the Oriental species. *Ibid.*, **31**, 127.

——— and CRAGG, F. W. (1913*a*). On certain haematophagous species of the genus *Musca*, with descriptions of two new species. *Ind. Jl. Med. Res.*, **1**, 11.

——— ——— (1913*b*). A new species of *Philaematomyia*, with some remarks on the genus. *Ibid.*, **1**, 26.

——— and EVANS, A. M. (1929). Insects, ticks, mites and venomous animals of medical and veterinary importance. Pt. I : Medical. Croydon.

——— and SENIOR-WHITE, R. (1924). The Oriental species of the genus *Musca* Linnaeus. *Rec. Ind. Mus.*, **26**, 553.

SNODGRASS, R. E. (1931). Morphology of the insect abdomen. Pt. I : General structure of the abdomen and its appendages. *Smithson. Misc. Coll.*, **85**, no. 6, publ. 3124.

——— (1933). Morphology of the insect abdomen. Pt. II : The genital ducts and the ovipositor. *Ibid.*, **89**, no. 8, publ. 3219.

——— (1935). Principles of insect morphology. (Chapter 19 : The organs of copulation and oviposition.) New York.

TOWNSEND, C. H. T. (1934). Manual of myiology. Pt. I : Development and structure. pp. 192, 199. São Paulo.

——— (1935). Manual of myiology. Pt. II : Muscoid classification and habits. pp. 134-40. São Paulo.

——— (1937). Manual of myiology. Pt. V : Muscoid generic diagnoses and data : Glossinini to Agriini. pp. 35-61. São Paulo.

ANALYSIS OF THE TRYPANOCIDAL ACTION OF TRIVALENT ARSENICALS AND ACRIFLAVINE

BY

FRANK HAWKING*

(From the Department of Pharmacology, Welsh National School of Medicine, Cardiff)

(Received for publication July 26th, 1938)

During recent years, it has been shown by Yorke *et al.* (1931), Jancsó (1931) and others that normal trypanosomes readily absorb trivalent arsenicals when exposed to dilute solutions of these compounds. Atoxyl-fast trypanosomes absorb them much less readily, and it is now considered that this explains the drug-resistance. The present paper describes an attempt to analyse the combination between drug and trypanosome in greater detail, and to investigate its relation to the eventual death of the parasite.

TRIVALENT ARSENICALS

TIME-ACTION CURVES. (*a*) *Heat.* As a preliminary step, investigations were made of the rate of death of trypanosomes (*T. rhodesiense*, Liverpool strain) following a physical injury, such as a brief exposure to heat. Trypanosomes were added to nutrient medium (1 part deactivated serum, 2 parts Locke-glucose) at 46° C.; after 4 minutes the tube was cooled and then incubated at 37° C. The number of surviving trypanosomes at various intervals is shown graphically in fig. 1. If the logarithm of the survivors be plotted graphically against time, the points approximate to a straight line (or a flat sigmoid curve), indicating a constant death-rate, as is common in these cases. Probably the physical agent (heat) sets up slow secondary changes, and the curve indicates the rate at which these changes occur in the different individual organisms. This experiment shows that, even when all the trypanosomes are exposed to the same physical injury, they do not all die at the same rate, and that, in the case of most of the organisms, there is but little interval between physical injury and death.

(*b*) *Arsenicals.* Determinations were then made of the rate of death of trypanosomes when exposed to suitable concentrations of trivalent arsenicals. The results of a typical experiment with reduced tryparsamide are shown graphically in fig. 2, A; experiments with other compounds—novarsenobillon, phenylarsenoxide and sodium arsenite—yielded similar results. When the logarithm of the survivors was plotted against the time, the later portion of each curve approximated to a straight line (fig. 2, B), suggesting a constant death-rate following an initial lag of about $1\frac{1}{2}$ hours. It will be shown below that this

* Gillson Scholar of the Society of Apothecaries of London.

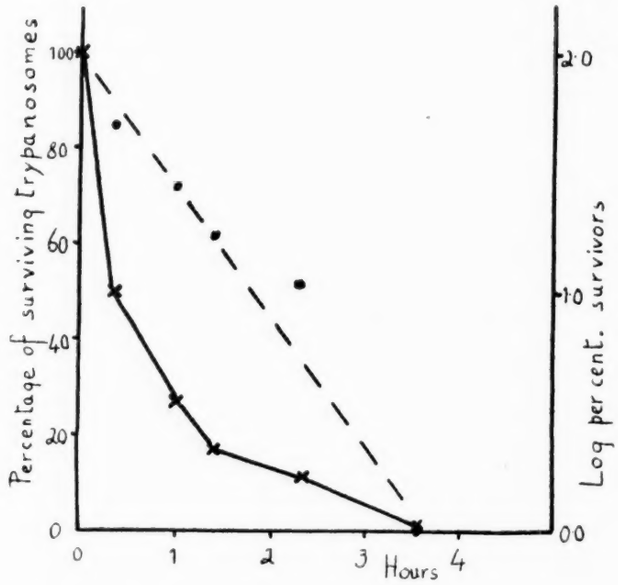


FIG. 1. Showing the death of trypanosomes following a short exposure to heat, viz., 4 minutes at 46° C.

Abscissa.—Time in hours; *Ordinate.*—Percentage of survivors.

Temperature, 37° C. Initial no. of organisms, 1,740 per mm³.

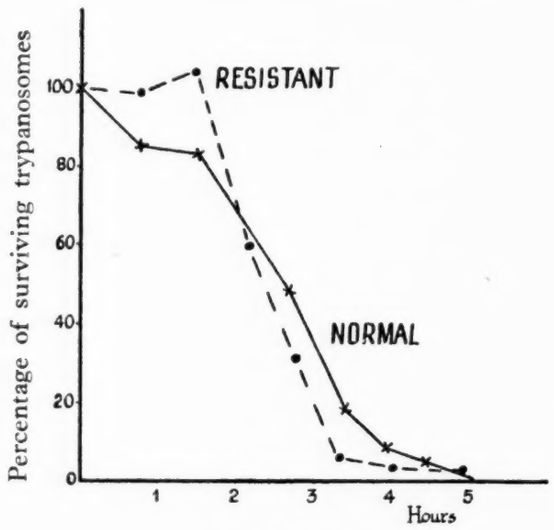


Fig. 2, A

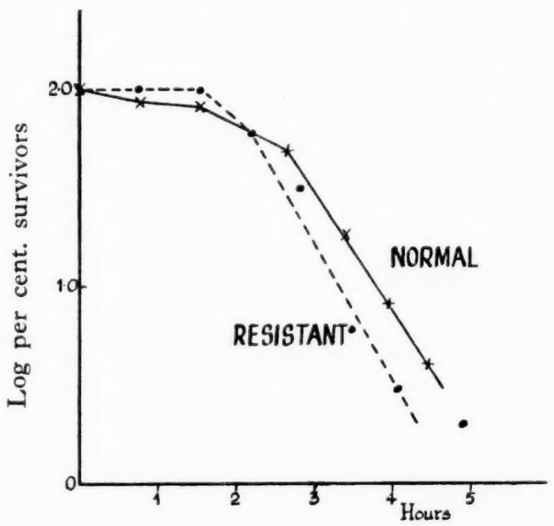


Fig. 2, B

FIG. 2, A. Time-action curves for the trypanocidal action of reduced tryparsamide.

Abscissa.—Time in hours; *Ordinate.*—No. of surviving trypanosomes per cent.; normal trypanosomes exposed to 0·078γ per ml.; resistant ones to 10γ per ml.

Temperature, 37° C. Initial no. of organisms, about 2,000 per mm³.

FIG. 2, B. Same as fig. 2, A.

Abscissa.—Time in hours; *Ordinate.*—Log percentage of survivors.

period of lag is much longer than the time required for the drug to be absorbed. The temperature-coefficient (Q/10) for this trypanocidal action is 1·8. The morphological changes accompanying the death of the organisms—swelling, distortion and eventually lysis—suggest that the drug causes the trypanosomes to

absorb water until finally they burst and disintegrate ; however, this swelling may only be secondary to some deeper cause. The fact that the curve for resistant trypanosomes is steeper than that for normal ones indicates that the former strain is the more homogeneous as regards the individuals that compose it.

Time-Concentration Curves. If the time-action curves for a number of different concentrations of a compound are constructed, and the points noted at which they cut the 10 per cent. survival line, a curve can be drawn showing the relation between the concentration and the time required to produce 90 per cent. mortality. The two extremities of such a curve tend to become parallel with the abscissa and ordinate respectively, indicating that there is a minimum concentration (C_o) of the drug required for trypanocidal action even with long exposure, and a minimum time (t_o) of exposure even with high concentrations. If the logarithm of the concentration minus minimum concentration, i.e., log $(C - C_o)$, be plotted against the logarithm of the reciprocal of the time, i.e., log $1/t$, (t_o being small enough to be disregarded), a straight line is obtained, according to the equation

$$n \log (C - C_o) = \log 1/t + \log k$$
$$\text{i.e., } (C - C_o)^n t = k$$

in which n and k are constants representing the slope and level respectively of the straight lines. Fig. 3 shows these curves plotted for reduced tryparsamide, phenylarsenoxide, novarsenobillon and sodium arsenite. This equation, $(C - C_o)^n(t - t_o) = k$, can be fitted to the time-concentration curves obtained from a great variety of pharmacological systems (Clark, 1933).

Fixation of Drug

An attempt was next made to analyse the trypanocidal action more closely. According to Clark (1933), ' the addition of a drug to a population of cells causes the following events : (1) fixation of drug by cells, (2) secondary chemical reactions between the drug and cell constituents, and (3) biological response to injury '— in this case, death of the trypanosomes. In the system under examination only the first stage, viz., fixation, is amenable to direct experiment by the present methods, and investigations were made of (*a*) the quantity, (*b*) the rate and (*c*) the reversibility.

(*a*) *Quantity of drug fixed.* Various suspensions of normal trypanosomes in nutrient medium were exposed to reduced tryparsamide 0·078γ per ml. for 50 minutes at 37° C. The tubes were then centrifuged, and the concentration of drug remaining in the supernatant fluid was estimated by testing its trypanocidal activity for fresh trypanosomes added to it in small numbers, according to the technique described by Yorke, Murgatroyd and Hawking (1931). The results of a typical experiment are given in Table I, which shows that a suspension of 16,000 trypanosomes per mm.^{3} removed 0·04γ of the drug, and that one of 59,000 trypanosomes per mm.^{3} removed 0·06γ.

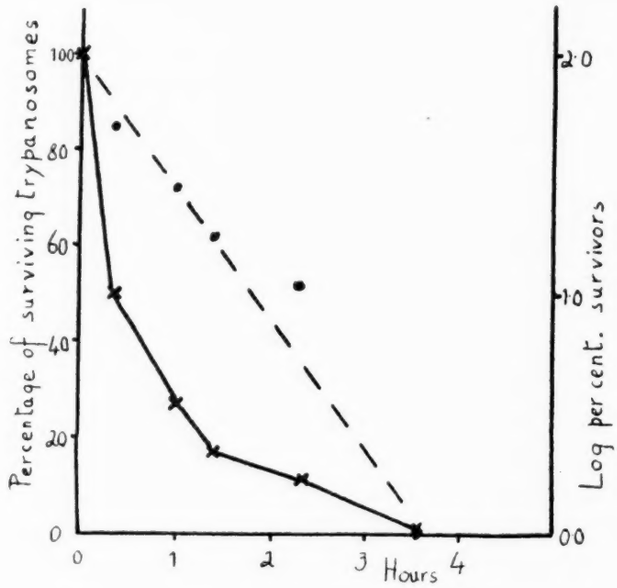


FIG. 1. Showing the death of trypanosomes following a short exposure to heat, viz., 4 minutes at 46° C.
Abscissa.—Time in hours; *Ordinate.*—Percentage of survivors.
Temperature, 37° C. Initial no. of organisms, 1,740 per mm³.

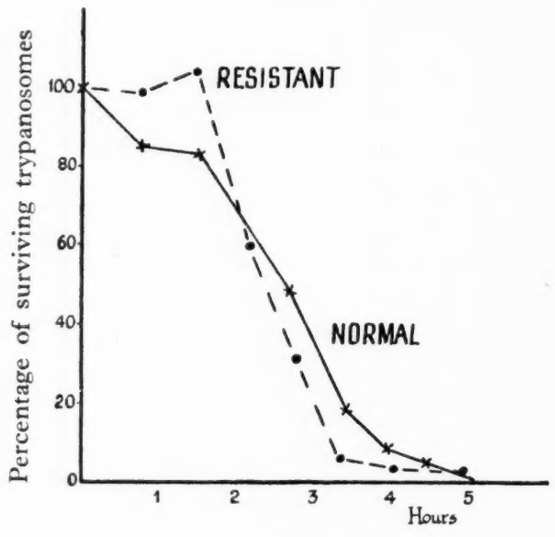


Fig. 2, A

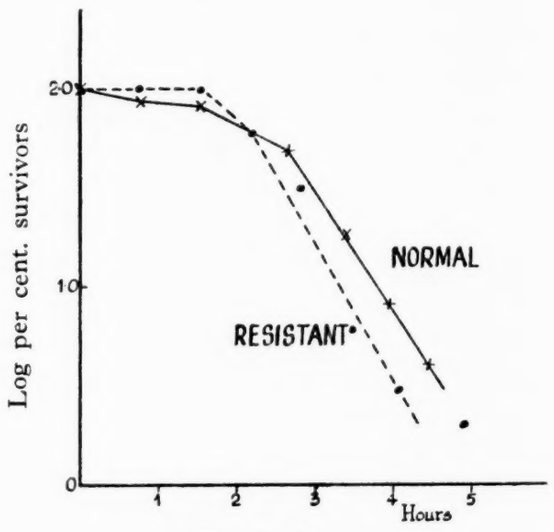


Fig. 2, B

FIG. 2, A. Time-action curves for the trypanocidal action of reduced tryparsamide.
Abscissa.—Time in hours; *Ordinate.*—No. of surviving trypanosomes per cent.; normal trypanosomes exposed to 0·078γ per ml.; resistant ones to 10γ per ml.
Temperature, 37° C. Initial no. of organisms, about 2,000 per mm³.

FIG. 2, B. Same as fig. 2, A.
Abscissa.—Time in hours; *Ordinate.*—Log percentage of survivors.

period of lag is much longer than the time required for the drug to be absorbed. The temperature-coefficient (Q/10) for this trypanocidal action is 1·8. The morphological changes accompanying the death of the organisms—swelling, distortion and eventually lysis—suggest that the drug causes the trypanosomes to

absorb water until finally they burst and disintegrate ; however, this swelling may only be secondary to some deeper cause. The fact that the curve for resistant trypanosomes is steeper than that for normal ones indicates that the former strain is the more homogeneous as regards the individuals that compose it.

Time-Concentration Curves. If the time-action curves for a number of different concentrations of a compound are constructed, and the points noted at which they cut the 10 per cent. survival line, a curve can be drawn showing the relation between the concentration and the time required to produce 90 per cent. mortality. The two extremities of such a curve tend to become parallel with the abscissa and ordinate respectively, indicating that there is a minimum concentration (C_o) of the drug required for trypanocidal action even with long exposure, and a minimum time (t_o) of exposure even with high concentrations. If the logarithm of the concentration minus minimum concentration, i.e., log $(C - C_o)$, be plotted against the logarithm of the reciprocal of the time, i.e., log $1/t$, (t_o being small enough to be disregarded), a straight line is obtained, according to the equation

$$n \log (C - C_o) = \log 1/t + \log k$$
$$\text{i.e., } (C - C_o)^n t = k$$

in which n and k are constants representing the slope and level respectively of the straight lines. Fig. 3 shows these curves plotted for reduced tryparsamide, phenylarsenoxide, novarsenobillon and sodium arsenite. This equation, $(C - C_o)^n(t - t_o) = k$, can be fitted to the time-concentration curves obtained from a great variety of pharmacological systems (Clark, 1933).

Fixation of Drug

An attempt was next made to analyse the trypanocidal action more closely. According to Clark (1933), ' the addition of a drug to a population of cells causes the following events : (1) fixation of drug by cells, (2) secondary chemical reactions between the drug and cell constituents, and (3) biological response to injury '— in this case, death of the trypanosomes. In the system under examination only the first stage, viz., fixation, is amenable to direct experiment by the present methods, and investigations were made of (*a*) the quantity, (*b*) the rate and (*c*) the reversibility.

(*a*) *Quantity of drug fixed.* Various suspensions of normal trypanosomes in nutrient medium were exposed to reduced tryparsamide 0·078γ per ml. for 50 minutes at 37° C. The tubes were then centrifuged, and the concentration of drug remaining in the supernatant fluid was estimated by testing its trypanocidal activity for fresh trypanosomes added to it in small numbers, according to the technique described by Yorke, Murgatroyd and Hawking (1931). The results of a typical experiment are given in Table I, which shows that a suspension of 16,000 trypanosomes per mm.^{3} removed 0·04γ of the drug, and that one of 59,000 trypanosomes per mm.^{3} removed 0·06γ.

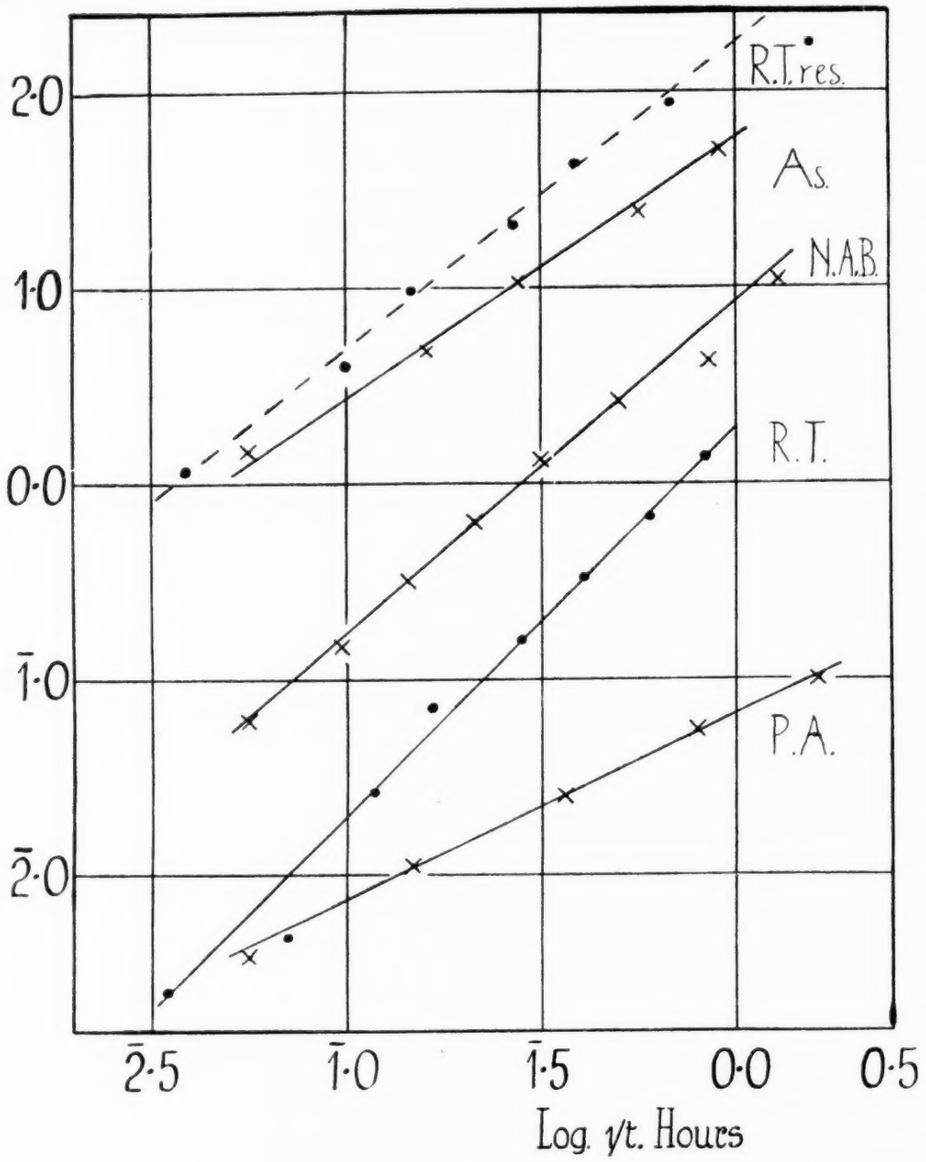


FIG. 3. Time-concentration curves for the trypanocidal action of reduced tryparsamide (R.T.), phenylarsenoxide (P.A.), novarsenobillon (N.A.B.), and sodium arsenite (As.) on normal trypanosomes ; and for reduced tryparsamide on resistant trypanosomes (R.T.res.).

Abscissa.—Log reciprocal of time required to produce 90 per cent. mortality, in hours ; *Ordinate.*—Log (C-C_0). Molar arsenic $\times 10^{-6}$.

Temperature, 37° C. Trypanosomes, about 1,300-2,000 per mm³.

For R.T.,	$C_0 = 0{\cdot}017$	As. molar $\times 10^{-6}$,		$n = 0{\cdot}50$, $k = 2{\cdot}04$
,, P.A.,	$C_0 = 0{\cdot}0036$	,,	,,	$n = 1{\cdot}04$, $k = 0{\cdot}016$
,, N.A.B.,	$C_0 = 0{\cdot}025$	,,	,,	$n = 0{\cdot}60$, $k = 3{\cdot}55$
,, As.,	$C_0 = 1{\cdot}77$	,,	,,	$n = 0{\cdot}77$, $k = 21{\cdot}9$
,, R.T.res.,	$C_0 = 1{\cdot}63$	,,	,,	$n = 0{\cdot}64$, $k = 27{\cdot}2$

From experiments of this type, and taking the volume of 100 million trypanosomes to be 2·4 mm.³ (experimental determination), it is possible to calculate the concentration of drug in (or on) the trypanosomes, and to compare it with that in the surrounding medium. Similar calculations can also be made in some

TABLE I

Showing the relation between (*a*) the number of trypanosomes, and (*b*) the amount of drug absorbed, when normal trypanosomes are exposed *in vitro* to reduced tryparsamide

Tube	Initial concentration of drug : γ per ml.	Trypanosomes per mm.^{3}	Concentration of drug in supernatant fluid : γ per ml.	Amount of drug absorbed by trypanosomes : γ
1	0·078	59,000	0·019	0·06
2	,,	30,000	0·019+	<0·06
3	,,	16,000	0·039	0·04
4	,,	8,300	0·039–0·078	? 0·02
5	,,	4,500	0·078—	?
6	0·078	Control	0·078	—
7	Control	59,000	—	—

Volume per tube, 1 ml. Temperature, 37° C. Period of exposure, 50 minutes.
In all tubes the trypanosomes were actively motile at the close of the exposure.

of the experiments described in a previous paper (Hawking, 1937), in which the amount of arsenic in the trypanosomes was determined chemically. From the experimental protocols, examples have been selected in which all the required data are available, and the results are given in Table II. The data there recorded show that the ' partition-ratio ' (i.e., concentration in trypanosome/concentration in medium) of reduced tryparsamide varies in the different experiments, but that for normal trypanosomes it is always high, the mean (geometric) of the series being 5,000 ; presumably much of the drug in or on the parasite must be present in an insoluble combined form. The partition-ratio for resistant trypanosomes is much lower, the mean (geometric) being 5 ; but it is still higher than unity, so that even in this case the results cannot be ascribed to a simple diffusion. The partition-ratio of phenylarsenoxide for normal trypanosomes is high, like that of reduced tryparsamide ; the data for resistant trypanosomes were inadequate, but they too indicated a high ratio. For arsenophenylglycine, sodium arsenite and tartar emetic, the partition-ratios are much lower, corresponding to the lower trypanocidal activity of these compounds *in vitro*. A more thorough investigation of the partition-ratio was made with acriflavine.

TABLE II

Showing the relation between the concentration of drug in the trypanosomes and that in the surrounding medium

Drug	Strain of trypano-somes	Initial concen-tration : γ per ml.	No. of try-panosomes per mm.^{3} $\times 10^5$	Final concentration : γ per ml.		Partition-ratio	Method of determination	
				In trypano-somes	In medium			
Reduced tryparsamide	Normal	1·25	1·5	340	0·03	11,000	Biological	
	,,	,,	2·0	260	<0·01	> 26,000	,,	(V)
	,,	,,	5·1	100	0·04	2,500	,,	(VI A)
	,,	0·62	1·5	170	0·02	8,300	,,	
	,,	0·078	0·24	77	0·03	2,600	,,	
	,,	,,	0·16	100	0·04	2,500	,,	(I)
	,,	,,	0·59	40	0·02	2,000	,,	(I)
	Resistant	40	2·7	290	38	7·6	Chemical	(VII B)
	,,	40	0·96	200	40	5	,,	
	,,	20	6·4	60	20	3	,,	(VII B)
Phenyl-arsenoxide	Normal	0·31	2·6	50	0·005	10,000	Biological	(VI A)
	,,	0·16	3·8	18	<0·0025	> 7,000	,,	
	,,	0·039	4·2	3·5	0·004	900	,,	(VI A)
Arsenophenyl-glycine	Normal	5·0	6·1	160	2·5	64	Biological	(VI A)
	,,	1·25	4·8	36	0·8	45	Chemical	(VII A)
	Resistant	5·0	8·3	120	2·5	48	Biological	(VI B)
	,,	1·25	7·7	12	1	12	Chemical	(VII B)
Arsenite*	Normal	0·625	1·9	43	0·42	100	Chemical	(VII A)
	Resistant	,,	2·7	50	0·32	160	,,	(VII B)
	,,	,,	4·8	8	0·52	15	,,	
	,,	,,	10	12	0·32	37	,,	
Tartar emetic	Normal	1·25	2·6	100	0·62	160	Biological	(VI A)
	Resistant	,,	2·2	150	0·45	300	,,	(VI B)

Temperature, 37° C. Duration of exposure, 15-50 minutes.

In all cases the trypanosomes were actively motile at the end of the exposure.

$$\text{Partition-ratio} = \frac{\text{concentration in trypanosomes}}{\text{concentration in medium}}$$

The figures in brackets in the final column refer to the tables in a previous paper (Hawking, 1937), except for I. which refers to the present paper.

* Expressed as arsenic trioxide.

From this ratio, the distribution of drug between trypanosomes and supernatant fluid can be calculated for any particular system. Thus it can be shown that a suspension of 1,500 normal trypanosomes per mm.^{3}, exposed to 0·08γ per ml. of reduced tryparsamide, as in fig. 2, would absorb about 15 per cent. of the total drug, while a similar one of resistant trypanosomes would absorb about 0·015 per cent.

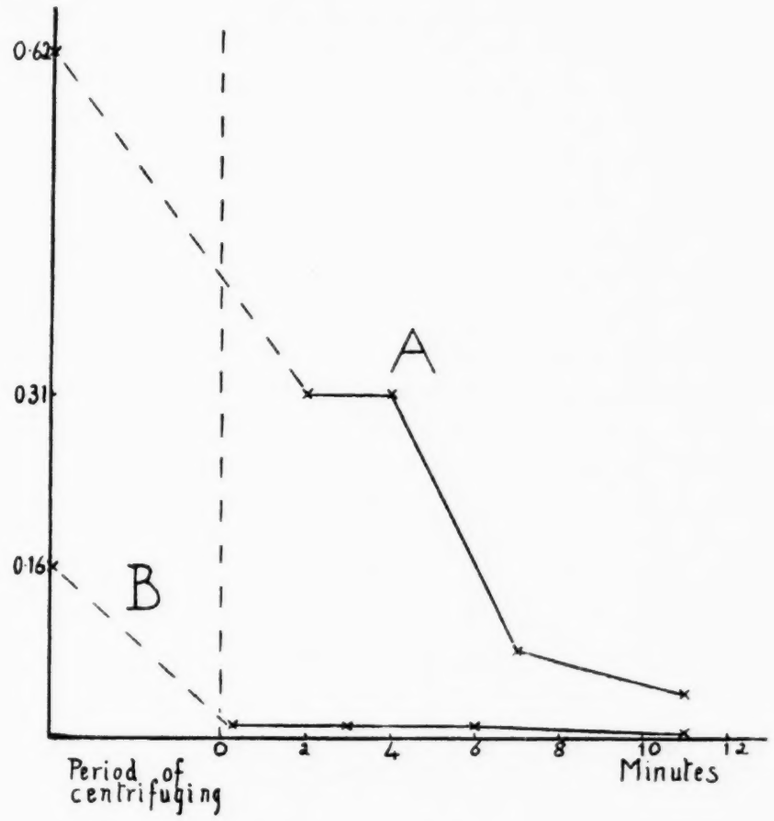


FIG. 4. Showing the rate of removal of reduced tryparsamide from the medium by a heavy suspension of normal trypanosomes.

Abscissa.—Time of contact between drug and trypanosomes, in minutes; *Ordinate.*—Concentration of drug in medium, γ per ml.

Temperature, 37° C.

A.—Initial concentration, 0·62γ per ml. Trypanosomes, 320,000 per mm^3.

B.—Initial concentration, 0·16γ per ml. Trypanosomes, 220,000 per mm^3.

(*b*) *Rate of fixation.* Experiments were made in which a heavy suspension of trypanosomes was mixed with a solution of the drug and allowed to remain in contact for varying periods of time. The mixture was then centrifuged to deposit the trypanosomes, and the trypanocidal activity of the supernatant fluid was determined in the usual way. As described in a previous paper (Hawking, 1937), it was found that the time required for combination to occur is short, and that it cannot be accurately determined, since a relatively long period (5 minutes) is required for the centrifugalization, during which period contact between drug

and trypanosome is inconstant and insusceptible of measurement. Thus, with reduced tryparsamide 0·16γ per ml. and excess of trypanosomes, e.g., 220,000 per mm.^{3}, the period of centrifugalization alone is sufficient for practically all the drug to be bound. With greater concentration of drug, e.g., 0·63γ per ml. and 320,000 trypanosomes per mm.^{3}, the process is not completed so quickly (fig. 4), although the rate of removal is probably higher. But even in this case 88 per cent. of the drug is bound by the trypanosomes in 7 minutes plus the period of centrifuging. Besides the time required for the reaction between the drug and the surface of the organism, the rate of absorption will also depend on the rate of diffusion of the compound through the medium (probably very important), and also on the rate of hydrolysis of the reduced tryparsamide, this compound being really a thioarsinite derivative, $H_2N.\ CO.\ CH_2.\ NH.\ C_6H_5.\ As = (S.\ CH_2.\ COONa)_2$, which undergoes reversible hydrolysis, liberating the arsenoxide.

(*c*) *Reversibility of fixation.* If the combination between drug and trypanosomes is reversible, then, after equilibrium has been reached, the presence of unabsorbed drug in the medium will be necessary to prevent the drug diffusing back out of the trypanosomes again ; and conversely.

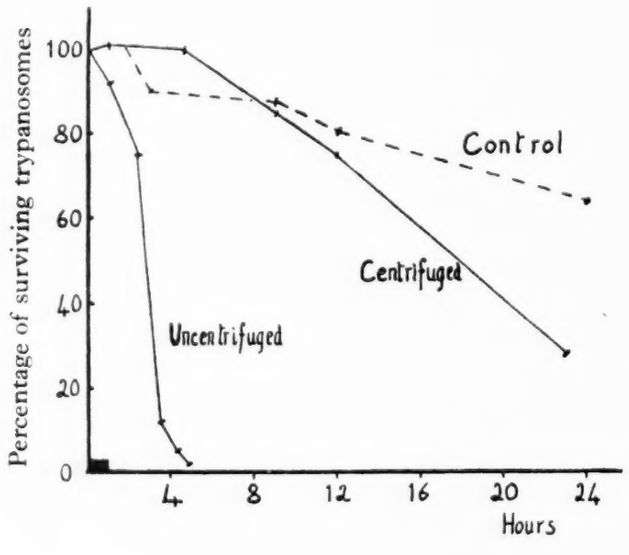


Fig. 5, A

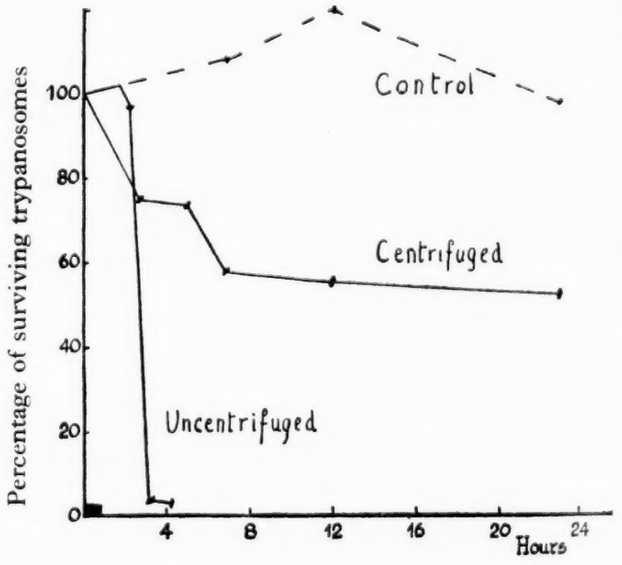


Fig. 5, B

FIG. 5, A. Showing the survival of normal trypanosomes after exposure to reduced tryparsamide, if centrifuged off and suspended in fresh medium.

Abscissa.—Time in hours ; *Ordinate.*—No. of survivors per cent.

Concentration of drug, 0·156γ per ml. Duration of exposure for the centrifuged tube, 1 hour.

Temperature, 37° C. Initial no. of trypanosomes, 2,500 per mm^3.

FIG. 5, B. Similar to fig 5, A, resistant trypanosomes.

Concentration of drug, 10γ per ml. Duration of exposure for the centrifuged tube, 50 minutes.

Initial no. of trypanosomes, 1,700 per mm^3.

EXPERIMENT. A series of parallel tubes was set up containing normal trypanosomes 2,000 per mm.^{3} and reduced tryparsamide 0·16γ per ml. These were incubated at 37° C. for various periods and then centrifuged ; the deposit of trypanosomes was washed once with dilute serum, and enough fresh medium was added to bring the volume back to its original dimensions. The survival of the trypanosomes was followed in the usual way, and the results were plotted as time-action curves. Fig. 5, A, shows the curves for selected tubes, viz., the tube not centrifuged, a tube centrifuged after 1 hour's exposure, and a control tube without drug, also centrifuged after 1 hour. The number of organisms in the centrifuged tubes is expressed as a percentage of the number found after washing and adding fresh medium. Fig. 5, B, records a similar experiment with resistant trypanosomes.

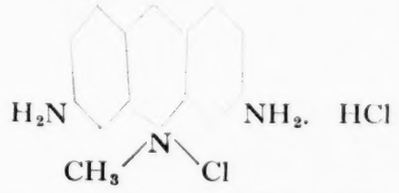
These curves show that the trypanosomes left in contact with the drug are almost all dead in 4 hours, but that most of those which had been exposed for 1 hour (during which period they had ample time to come into equilibrium with the environment), and then washed, lived almost as well as the trypanosomes in the control tubes. Other experiments showed that the duration of contact between drug and trypanosomes did not greatly affect their subsequent survival, provided that the exposure was not so long that many of the organisms were already beginning to die during this stage of the experiment. Clearly the drug which has been fixed by the trypanosomes in the first part of the experiment has been washed out again during the second, and the fixation is reversible. Since under these conditions it continues to be reversible during the whole of the first hour, the early part of the second phase of drug-action, viz., secondary chemical reactions between drug and cell-constituents, must also be reversible; otherwise the trypanosomes would not recover when removed from contact.

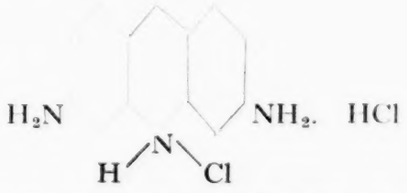
ACRIFLAVINE

The trypanocidal action of acriflavine is so similar to that of the trivalent arsenical compounds that it is usually considered to act on the same ' receptor.' Since it is susceptible of colorimetric estimation and also induces photosensitivity, it permits closer analysis of the process of fixation.

Chemically, acriflavine has usually been considered to be a pure compound, 2 : 8 diamino-10 methyl-acridinium-chloride hydrochloride (formula I) ; but towards the end of this work my attention was drawn to a paper by Gailliot (1934), which showed that the pharmaceutical preparations of acriflavine, such as had been here used,

I





II

really consisted of a mixture of this with about 25-40 per cent. of 2 : 8 diamino-acridinium-chloride hydrochloride (formula II). Dr. A. J. Ewins kindly supplied pure specimens of these two compounds, the properties of which were then compared. In solution, the fluorescence and colour of the hydrochloride (II) are only about half those of the methochloride (I). Oesterlin (1936) found that, when the methochloride was exposed to ultra-violet light in solution at pH 6·0, it emitted light of wavelengths 460-640$\mu\mu$, maximum at

490$\mu\mu$; while the diamino-acridine (II) emitted fluorescence of the same wavelengths but only half the intensity. The trypanocidal activity of the hydrochloride is slightly less than that of the methochloride, the minimum trypanocidal concentration at 37° C. being about 0·16γ per ml. at 7 hours and 0·078γ per ml. at 24 hours for the former, and 0·16γ per ml. at 7 hours and 0·039γ per ml. at 24 hours for the latter. The hydrochloride is less active in inducing photosensitivity ; thus the ' light-index ' of trypanosomes exposed to 0·078γ per ml. of the hydrochloride is 15 seconds, to 0·039γ per ml. 48 seconds, and to 0·019γ per ml. 116 seconds ; while that of trypanosomes exposed to 0·039γ per ml. of the methochloride is 7 seconds, and to 0·019γ per ml. 12 seconds. The hydrochloride is also less avidly absorbed by trypanosomes ; in one experiment the concentration in the trypanosome was only 1,700 times that in the surrounding fluid, while in the case of the methochloride it was 4,700 times as great (Table III, A).

Thus the activity of acriflavine is due mostly to that of the 2 : 8 diamino-10 methyl-acridinium-chloride hydrochloride (I) diluted by the presence of 25-40 per cent. of the less active 2 : 8 diamino-acridinium-chloride hydrochloride (II). In the rest of the paper acriflavine has been treated as a single substance, possessing the properties of such a mixture, as this does not materially affect the general argument.

Technique. Acriflavine in solution was measured in three ways :

(*a*) By colorimeter, using an instrument of the Duboscq pattern ; this is suitable for concentrations of 1-10γ per ml.

(*b*) By fluorescence (Hawking, 1934), which measures down to 0·2γ per ml. in dilute serum, and 0·04γ per ml. in alcohol.

(*c*) By photosensitivity, i.e., small numbers of fresh trypanosomes are added to the supernatant fluid and their photosensitivity is determined as will be described in a subsequent paper. This method is of low accuracy, but it enables concentrations as dilute as 0·001γ per ml. to be measured.

Acriflavine in the trypanosomes was extracted with absolute alcohol, two lots of 4 ml. being used. Further extraction was done with 4 ml. absolute alcohol containing N/100 hydrochloric acid, and the tube was warmed to 50° C. ; by this means complete extraction of all but small traces was obtained. The acriflavine content of the various extracts was then determined separately by comparison with solutions of the dye in alcohol or acid alcohol as the case might be.

Time-Action and Time-Concentration Curves. Time-action curves can be obtained for acriflavine similar to those for reduced tryparsamide. Such curves are less steeply sloped, however, indicating that there is a greater range in sensitivity among the individual trypanosomes to acriflavine than there is in that to arsenicals. The morphological changes produced are similar, but they appear earlier and there is a longer delay after death (immobilization) before the organisms disintegrate. Time-concentration curves may also be obtained as before. Taking the time required to kill 60 per cent. of the organisms as the most convenient period to measure, and expressing concentrations as molar $\times 10^{-6}$ (M.W. = 216), the equations for typical experiments are :

For normal trypanosomes $(C\text{-}0{\cdot}15)^{0{\cdot}64}\, t = 1{\cdot}44$

For resistant trypanosomes $(C\text{-}2{\cdot}3)^{0{\cdot}78}\, t = 51{\cdot}3$

Fixation of Acriflavine

(*a*) *Rate.* In a previous paper (Hawking, 1934) it was shown that fixation occurs rapidly both at 37° C. and 16° C., a few minutes sufficing for equilibrium to be reached.

(*b*) *Quantity fixed and partition-ratio.* The following is a typical experiment.

Tubes were set up containing trypanosomes and acriflavine in a medium consisting of 1 part serum plus 11 parts Locke-glucose solution. After standing for 20 minutes in the dark, the tubes were centrifuged and the amount of acriflavine present in the deposits and in the supernatant fluids was estimated colorimetrically. The results of this experiment are shown in Table III, A.

TABLE III, A

Showing the absorption of acriflavine, diamino-acridine, and diamino-methyl-acridine by normal trypanosomes

Tube	Drug	Total drug present : γ	Final condition of trypanosomes	Drug in trypanosomes		Drug in supernatant fluid		Partition-ratio
				Total : γ	Concentration : γ per ml.	Total : γ	Concentration : γ per ml.	
1	Acriflavine	1,280	Non-motile	427	29,700	533	178	167
2		640	Non-motile	347	24,100	133	44·3	545
3		320	Half motile	305	15,800	15·5	3·87	4,080
4		160	Mostly motile	155	8,050	5·5	1·37	5,870
5		80	Motile	76	3,960	4	1·0	3,960
6	Diamino-methyl-acridine	160	Motile	153	7,970	6·8	1·7	4,690
7	Diamino-acridine	160	Motile	143	7,420	17·5	4·37	1,700

Volume per tube, 4 ml. Temperature, 18° C. Duration of exposure, 20 minutes.
No. of trypanosomes per tube, $8{\cdot}0 \times 10^8$. Volume of trypanosomes per tube, 19·2 mm.^{3}
This experiment is shown by ⓥ in fig. 6.

A similar experiment was performed with resistant trypanosomes (Table III, B); in this case the results are based on the amount of acriflavine extracted from the trypanosomes. In all these experiments, washing of the deposits has been purposely avoided on account of the reversibility of the combination between drug and cells (see below).

Microscopically, the normal trypanosomes were seen to be stained while actively motile ; the resistant ones did not show obvious staining until after immobilization. Immobilized organisms of both types had a similar appearance. The cytoplasm was stained bright yellow and contained a few fine brown granules ; the nucleus was not discernible ; in some trypanosomes a small brown granule could be seen with difficulty in the position of the parabasal body.

TABLE III, B

Showing the absorption of acriflavine by living and dead resistant trypanosomes

Tube	Total acriflavine present : γ	Final condition of trypanosomes	Drug in trypanosomes		Drug in supernatant fluid		Partition-ratio
			Total extracted : γ	Concentration : γ per ml.	Total calculated : γ	Concentration : γ per ml.	
1	5,120	Non-motile	650	26,500	4,470	1,120	21·7
2	2,560	Non-motile	103	4,210	2,460	615	6·84
3	1,280	Some feebly motile	57·7	2,350	1,220	305	7·67
4	640	Mostly motile	25·4	1,040	615	154	6·75
5	320	Motile	14·6	596	305	76·2	7·82
6	160	Motile	9·5	388	150	37·5	10·4
7	160	(Killed before commencement)	85·7	3,500	74·3	18·6	187

Volume per tube, 4 ml. Temperature, 19° C. Duration of exposure, 23 minutes.
No. of trypanosomes per tube, $10{\cdot}9 \times 10^8$. Volume of trypanosomes per tube, 24·5 mm.^{3}
This experiment is shown by + (resistant) in fig. 6.

Tables III, A and B, show that, when exposed to concentrations of less than 80γ per ml. (tube 3A), this suspension of normal trypanosomes absorbs practically all the acriflavine present, the ratio between the concentrations inside and outside the trypanosomes respectively (partition-ratio) being approximately constant ; with concentrations of acriflavine greater than this, the trypanosomes approximate to complete saturation with the dye. Almost complete absorption occurs even in tubes (e.g., 5A) in which the motility of the trypansosomes is not impaired. Diamino-methyl-acridinium hydrochloride is more avidly absorbed than diamino-acridinium hydrochloride, and it has a higher partition-ratio. Resistant trypanosomes, when exposed to concentrations of less than 320γ per ml. (tube 3B), absorb only a small proportion of the acriflavine present, and the partition-ratio, which is approximately constant, is low but yet greater than unity. With high

concentrations, which cause death of the organisms during the experiment, the relative absorption of the dye is much increased (tube 1B). Dead resistant trypanosomes (tube 7B) absorb much more of the dye than living ones (cf., the greater absorption of arsenicals by dead resistant trypanosomes, Hawking, 1937); and this suggests that the increased absorption in tube 1B is due to the death of the organisms. However, in another experiment (shown by white squares in fig. 6), in which resistant trypanosomes were exposed to 640γ per ml., and in which most of the organisms maintained their motility, the intracellular concentration of dye was nevertheless as high as 8,000γ per ml. Comparison of tube 7B with tube 4A shows that, even when dead, resistant trypanosomes do not absorb as avidly as living normal ones do, the partition-ratio in the two cases being markedly different.

The results of a large number of experiments on normal and resistant trypanosomes over a wide range of concentrations of acriflavine are shown graphically in fig. 6. The experiments were performed at 16-20° C. and the period of exposure was 20-40 minutes. Normal trypanosomes, in which the intracellular

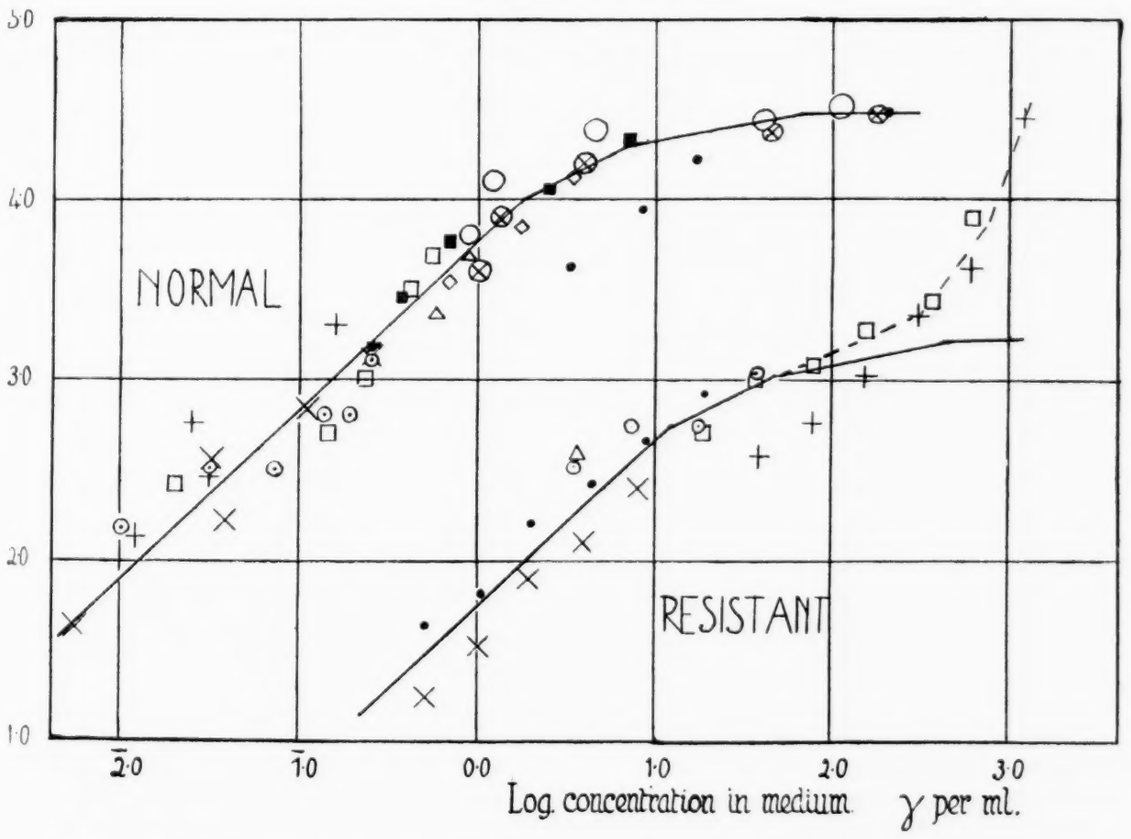


FIG. 6. Showing the relation between the concentration of acriflavine in the trypanosomes and that in the surrounding medium.

Abscissa.—Log concentration in the medium, γ per ml.; *Ordinate.*—Log concentration in the trypanosomes, γ per ml. The various signs refer to different experiments, normal and resistant trypanosomes being examined on different days. For details, see text.

concentration was more than 16,000γ per ml. (log 4·2), were mostly immobile at the end of the experiment; those in which the internal concentration was lower remained motile. Resistant trypanosomes remained motile except in the instances shown in Table III, B. The figure shows that, although the points for normal trypanosomes are a little scattered, in the lower concentrations they approximate to a line at an angle of 45°, i.e., the internal concentration is directly proportional to the external concentration. With the higher external concentrations, the graph of the internal concentration approaches an asymptote of about log 4·5, i.e., 32,000γ per ml. The death of the parasites with internal concentrations of more than log 4·2 does not cause any upward flexion of the general curve, such as would accompany increased absorption. Theoretically, it is possible that the amount of acriflavine absorbed (y) depends upon the concentration in the environment (x) and upon the number of receptors still free to combine with the drug (B—y),

$$\text{e.g., } kx \quad \frac{y}{B - y}$$

i.e., Langmuir's formula for adsorption, when one molecule of drug combines with one receptor (x = concentration in supernatant fluid γ per ml., y = concentration in trypanosomes γ per ml., B = concentration in trypanosomes γ per ml., when saturation is complete). The curve in fig. 6 has been drawn to pass as close to as many of the points as possible, B being taken as 32,000, and k as 0·252. This equation is seen to give an approximate expression to the relation experimentally observed, but it is not safe to draw any theoretical conclusion from it. Empirically, the equation is of value because, when y is low compared with B, i.e., with the lower concentrations of acriflavine, the curve becomes a straight line and the equation can be written $kx = \frac{y}{B}$ i.e., $\frac{y}{x} = kB = 8{,}050$, which is the partition-ratio. Since the volume of a million trypanosomes is 0·024 mm.^{3}, and the sum of the amounts of acriflavine in the trypanosomes and fluid respectively is equal to the total amount originally present, it can be calculated that, when a suspension of 1,500 trypanosomes per mm.^{3} is exposed to low concentrations of the compound (cf., fig. 2), equilibrium is reached when the organisms here absorbed 22 per cent. of the total amount present.

The curve for the resistant trypanosomes is less easy to interpret, especially as the points are more scattered. The curve for the lower concentrations is roughly parallel with that for normal trypanosomes, but, as the very high concentrations are reached, the curve turns sharply upwards, the highest intracellular concentration recorded being as high as that for normal trypanosomes, although corresponding to an extracellular concentration six times as great. The simplest interpretation is that living resistant trypanosomes follow an equation similar to

that for normal ones, the maximum (hypothetical) internal concentration (B) being about 1,760γ per ml., and k being 0·035 ; the absorption of these amounts, however, rapidly leads to the death of the trypanosomes, whose powers of absorption are then much increased, thus explaining the change of direction in the later portion of the experimental curve. As with normal trypanosomes, when y is small compared with B, the equation can be written $y/x = kB = 61$, which is the partition-ratio. This value of the ratio has an empirical basis, and it is really independent of the hypothesis from which it has been derived. By calculation as above, it can be deduced that, when resistant trypanosomes, 1,500 per mm.^{3}, are suspended in low concentrations of acriflavine, they absorb 0·22 per cent. of the drug present.

(*c*) *Reversibility of fixation.* The reversibility of the combination between trypanosome and acriflavine was investigated by exposing trypanosomes to the drug and then subjecting them to repeated washings. Between each washing they were incubated for 10 minutes at 37° C. At each stage the photosensitivity

TABLE IV

Showing the washing-out of acriflavine from trypanosomes

A. NORMAL TRYPANOSOMES

Initial concentration of acriflavine, 0·156 γ per ml. Initial no. of trypanosomes, 1,600 per mm^3.

Tube	No. of washings	Average light-index of trypanosomes in seconds	Corresponding acriflavine-content of trypanosomes : γ per 10^9	No. of trypanosomes per mm.^{3} surviving after 24 hours
1	0	4·9	22	0
2	1	18·5	6·6	0
3	2	24	5·1	180
4	3	26	4·8	580
5	4	73	2·0	420

B. RESISTANT TRYPANOSOMES

Initial concentration of acriflavine, 2·5γ per ml. Initial no. of trypanosomes, 1,600 per mm.^{3}

1	0	3·4	30	0
2	1	20	6·2	320
3	2	48	2·9	830
4	3	100	1·5	450
5	4	126	0·8	—

Temperature, 37° C. Light-index = Lethal period of illumination, on stage of dark-ground microscope under standard conditions.

of the organisms was determined, and the corresponding acriflavine-content was obtained from the empirical equations to be described in a subsequent paper, viz., for normal trypanosomes, $1{\cdot}12 \log A = \log P + 0{\cdot}21$; for resistant trypanosomes, $1{\cdot}12 \log A = \log P + \bar{1}{\cdot}87$, where A = acriflavine-content of trypanosomes γ per 10^9, and P = 100/light-index, i.e., lethal period of illumination in seconds. The results of these experiments are given in Table IV, which shows that with the first washing the photosensitivity, and so presumably the acriflavine-content of the normal trypanosomes, is much diminished. Subsequent washings remove decreasing quantities of drug, as might be expected. With resistant trypanosomes, washing-out occurs more readily ; presumably the dye is bound less firmly. These experiments confirm those previously described for the washing-out of reduced tryparsamide ; with both compounds it is clear that fixation is reversible.

DISCUSSION

As already mentioned, Clark divides the pharmacological action of drugs into the three stages of (1) fixation of drugs by cells, (2) secondary chemical reactions between drug and cell-constituents, and (3) biological response to injury. This analysis forms a useful framework for the present discussion.

(*a*) *Fixation.* Most of the evidence accumulated in this work deals with fixation ; it has been shown to be rapid (reaching equilibrium in a few minutes) and to a large extent reversible.

The reversibility of fixation and its proportionality to the concentration in the surrounding fluid were inferred by Singer (1936) from the different behaviour of trypanosome suspensions diluted with normal plasma from that of those diluted with plasma of the treated animal. Pedlow and Reiner (1935) did not detect any reversibility in the binding of neoarsphenamine, but their methods were not sufficiently delicate to estimate the very small quantities involved. Since fixation is reversible and proportional, the drug-content of the trypanosomes exposed to a compound *in vivo* mirrors that of the animal's plasma, and diminishes in proportion to the time elapsed after the injection, as was shown by Hasskó (1935) using direct measurement, and by Jancsó (1931) observing the photosensitivity. The rapidity and reversibility of the reaction are comparable to those described by Christophers (1929) during the haemolysis produced by acid or alkali ; large amounts of the acid were fixed by the cells in a few minutes, but haemolysis did not occur until after a latent period, inversely proportional (within limits) to the concentration of acid or alkali used ; if the erythrocytes were suspended in fresh saline, acid diffused out of the cells into the medium, until equilibrium was again established. Another parallel of rapid fixation followed by delayed action is found in the case of mercuric chloride ; if bacteria are exposed to this and then centrifuged, the washing deposit contains combined mercury, turning black on the addition of a soluble sulphide ; if the addition of this antidote (sulphide) is not too long delayed, the bacteria will revive and prove capable of multiplication when transferred to a suitable nutrient medium (Chick, 1908).

It has been shown that the absorption of acriflavine follows certain laws and that there is a definite quantitative relationship between the amount in, or on, the trypanosomes and that in the surrounding fluid. The same appears to be the case with the trivalent arsenicals. The expression of these quantities in terms of molecules is of interest. Thus, when a suspension of trypanosomes, 3,000 per

mm.^{3}, is exposed to acriflavine 0·63γ per ml., most of the organisms die in about 5 hours. About 44 per cent. of the drug would be absorbed by this number of trypanosomes, which would correspond to $9{\cdot}2 \times 10^{-8}\gamma$ per trypanosome. Assuming that 1 gm.mol. contains $6{\cdot}4 \times 10^{23}$ molecules, and that the area covered by an adsorbed molecule of acriflavine is about the same as that covered by an adsorbed molecule of methylene blue, viz., 5×10^{-7} sq. μ (Paneth and Radu, quoted by Clark, 1933), it may be shown that this quantity is equivalent to $2{\cdot}7 \times 10^8$ molecules, which would form a monomolecular film of 140 sq. μ. The surface area of a trypanosome, measured as a cylinder 25μ long and 1·5μ in diameter, is about 120 sq. μ ; thus the quantity of acriflavine required to kill a trypanosome under these conditions is sufficient to form a monomolecular film over its whole surface. However, this similarity of dimension is probably only a coincidence, since Jancsó (1932) showed that the acriflavine is accumulated mostly in the blepharoplast and, to a lesser extent, in the nucleus. The maximum possible concentration in trypanosomes is 32,000γ per ml. (fig. 6), which corresponds to $7{\cdot}7 \times 10^{-7}\gamma$, i.e., $2{\cdot}3 \times 10^9$ molecules per trypanosome, which would cover 1,100 sq. μ. Similar calculations for reduced tryparsamide may be based on the time-action curve of fig. 2, in which 2,000 trypanosomes per mm.^{3} were exposed to 0·078γ per ml. These organisms would absorb about 20 per cent. of the drug present, which would allow $7{\cdot}8 \times 10^{-9}\gamma$, i.e., $1{\cdot}1 \times 10^7$ molecules per trypanosome, a quantity which killed most of them in 3 hours ; this amount would form a monomolecular film of 5·5 sq. μ, i.e., only about 5 per cent. of the surface of the trypanosome.

The minimum trypanocidal concentration (after 24 hours' exposure) of phenylarsenoxide is $1{\cdot}4 \times 10^{-8}$ molar, of reduced tryparsamide $2{\cdot}2 \times 10^{-8}$ molar, and of acriflavine $1{\cdot}8 \times 10^{-7}$ molar. These figures may be compared with the minimum effective concentrations (collected by Clark, 1933) for adrenaline or histamine to cause vaso-constriction in the rabbit's ear, viz., 5×10^{-10} molar, or for acetylcholine to inhibit the frog's heart, viz., 10^{-9} molar ; in these cases the cells are only influenced and not killed.

(*b*) *Secondary chemical reactions.* These appear to take up most of the time required for trypanocidal action, but very little is known about them. It seems probable (fig. 5) that the earlier parts are reversible ; and the temperature-coefficient is 1·8. Perhaps manometric studies of the type described by Fulton and Christophers (1938) may elucidate this phase.

(*c*) *Biological response to injury.* As described above, the actual death of the trypanosomes is preceded by a short period in which the organisms are swollen and distorted. In the experiment of fig. 2, this phase probably lasted about half an hour. With equivalent concentrations of acriflavine, the period of swelling is more prolonged. Although the relation between the distortion and the dying of the trypanosome cannot be definitely stated, it seems probable that the swelling and eventual disintegration are secondary to some more fundamental injury, especially as similar morphological changes and time-action curves are obtained following exposure to heat.

The nature of drug-resistance in trypanosomes. The work of Yorke *et al.* (1931), Jancsó, Singer and others, to show that drug-resistance is due to dimished fixation, has been reviewed at length in a previous paper (Hawking, 1937). The present work helps to estimate the extent of this diminution, although the mechanism by which it is produced is still obscure. Whether the processes subsequent to fixation are the same in resistant as in normal trypanosomes is not easy to decide. Evidence has been sought by using the partition-ratios to calculate the intracellular concentration of drug necessary to kill trypanosomes in six hours. Taking the average for three experiments with each strain, the initial concentration of acriflavine required to kill normal trypanosomes (2,400 per mm.^{3}) in six hours was 0·33γ per ml., and to kill resistant ones, 5·7γ per ml. With normal trypanosomes the final concentration in the medium would be 30 per cent. less than this, owing to absorption by the organisms. Then, taking the partition-ratios as 8,000 and 60 respectively, the intracellular concentration to kill normal trypanosomes in six hours would be 1,700γ per ml. and to kill resistant ones 350γ per ml. Similar calculations for reduced tryparsamide, based on fig. 2, yield values of 104γ per ml. for normal trypanosomes and 24γ per ml. for resistant ones. The result obtained in both cases, that normal trypanosomes require four to five times as much drug to produce death as resistant ones do, is surprising. Perhaps in the former case a large proportion of the drug is diverted into inactive combinations.

SUMMARY

1. Time-action curves for the trypanocidal action of trivalent arsenicals and acriflavine show that there is a constant death-rate, following an initial lag which is much longer than the time required for fixation of the compound.

2. Time-concentration curves show that the trypanocidal action follows the equation $(C - C_o)^n t = k$, where n is 0·5–0·8, except in the case of phenylarsenoxide (1·04).

3. From analogy with other pharmacological processes, it is probable that the reaction between drug and trypanosome occurs in three stages, viz., fixation of the drug, secondary chemical reactions, and eventual death of the organism. The fixation occurs rapidly, being complete in a few minutes, and is reversible. The earlier part of the secondary chemical reactions is also reversible. The temperature coefficient (Q/10) of the whole trypanocidal process is 1·8.

4. When equilibrium has been reached between drug inside and outside the trypanosome, the partition-ratio (i.e., concentration inside trypanosome/concentration outside trypanosome) of reduced tryparsamide is about 5,000 for normal trypanosomes and 5 for resistant ones ; for phenylarsenoxide it is about 10,000, for arsenophenylglycine about 50, for sodium arsenite 30-100, and for tartar emetic about 200.

5. The relation between the concentrations of acriflavine inside and outside normal trypanosomes respectively approximates to Langmuir's equation

for adsorption, viz., $kx = \frac{y}{B - y}$. With low concentrations this gives a constant ratio (partition-ratio) between the internal and external concentrations respectively, viz., 8,000. When normal trypanosomes are saturated with acriflavine they contain drug equivalent to 3 per cent. of their own (moist) weight.

6. The absorption of acriflavine by resistant trypanosomes shows a similar relationship with the lower concentrations, but the partition-ratio is only 60. With higher concentrations the relationship is distorted by the fact that the death of resistant trypanosomes is accompanied by an increase in their powers of absorption.

7. The amount of reduced tryparsamide required to kill a normal trypanosome in three hours is about $8 \times 10^{-9}\gamma$, which is sufficient to form a monomolecular film over only 5 per cent. of its surface. The corresponding amount of acriflavine is $9 \times 10^{-8}\gamma$, which is sufficient to cover the whole surface.

ACKNOWLEDGEMENTS.—This work was assisted by a grant from the Medical Research Council. Acknowledgements are due to Professor A. J. Clark for advice on the interpretation of some of the curves, to Dr. A. J. Ewins, of Messrs. May and Baker, for supplying many of the compounds used and for information about them, and to Professor Warrington Yorke for the strain of trypanosomes.

REFERENCES

CHICK, H. (1908). An investigation of the laws of disinfection. *Jl. Hyg., Camb.*, **8,** 92.

CHRISTOPHERS, S. R. (1929). The mixture reaction in haemolysis by acids and bases. *Indian Jl. Med. Res.*, **17,** 533.

CLARK, A. J. (1933). The mode of action of drugs on cells. Lond.

FULTON, J. D., and CHRISTOPHERS, SIR S. R. (1938). The inhibitive effect of drugs upon oxygen uptake by trypanosomes (*Trypanosoma rhodesiense*) and malaria parasites (*Plasmodium knowlesi*). *Ann. Trop. Med. & Parasitol*, **32,** 77.

GAILLIOT, M. (1934). Composition and solubility of acriflavine and some other derivatives of 2, 8-diaminoacridine used in therapeutics. *Quart. Jl. Pharm.*, **7,** 63.

HASSKÓ, A. (1935). Experimentelle Beiträge zur Wirkungsweise chemotherapeutischer Mittel, I. *Ztschr. f. Hyg. u. Infekt.*, **116,** 660.

HAWKING, F. (1934). The absorption of acriflavine by trypanosomes. *Ann. Trop. Med. & Parasitol.*, **28,** 67.

——— (1937). Studies on chemotherapeutic action. I: The absorption of arsenical compounds and tartar emetic by normal and resistant trypanosomes and its relation to drug-resistance. *Jl. Pharmacol.*, **59,** 123.

JANCSÓ, N. von (1931). Photobiologische Studien in der Chemotherapie. I: Lichtempfindliche Trypanosomen in trypaflavinbehandelten Tieren. *Zentralbl. f. Bakt., Orig.*, **122,** 388.

——— (1932). Beobachtung chemotherapeutischer Vorgänge im Fluorescenzmikroskop. *Klin. Woch.*, **11,** 689.

OESTERLIN, M. (1936). Zur Chemotherapie der Infektionskrankheiten. *Ztschr. f. Hyg. u. Infekt.*, **118,** 263.

PEDLOW, J. T., and REINER, L. (1935). On the mechanism of chemotherapeutic action. XII: Comparison of the binding of chemotherapeutic agents by normal and resistant trypanosomes. *Jl. Pharmacol.*, **55,** 179.

SINGER, E. (1936). Die Wirkung der Chemotherapeutica auf die Trypanosomenzelle. *Ztschr. f. Hyg. u. Infekt.*, **117,** 752.

YORKE, W., MURGATROYD, F., and HAWKING, F. (1931). Studies in chemotherapy. V: Preliminary contribution on the nature of drug resistance. *Ann. Trop. Med. & Parasitol.*, **25,** 351.

PRINTED IN GREAT BRITAIN BY H. R. GRUBB, LTD., CROYDON.

UNIVERSITY OF LIVERPOOL JAN 10 1939

1st December, 1938 Vol. 32. No. 4

MEDICAL LIBRARY

Annals of Tropical Medicine and Parasitology

ANNALS
Edited by
Prof. W. Yorke
Prof. D. B. Blacklock
Prof. R. M. Gordon
Dr. T. Southwell
Prof. T. H. Davey

ISSUED BY
THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE

THE UNIVERSITY PRESS OF LIVERPOOL

Price Seven Shillings and Sixpence Net

Essential

to effective

leprosy contro

IODISED

— 'MOOGROL' —

A Mixture of Esters of Acids of the Chaulmoogric Series with 0·5 per cent. Iodine

Relatively free from general toxic effects. Suitable for intradermal and intramuscular injection

Bottles of 25 c.c.

Bottles of 50 c.c.

'MOOGROL'

Mixture of Esters of Acids of the Chaulmoogric Series

Bottles of 25 c.c.

Bottles of 50 c.c.

BURROUGHS WE
LON

ARCUS SENILIS AS A SIGN OF CHRONIC VITAMIN C DEFICIENCY

BY

N. L. CORKILL, M.D.

(*Sudan Medical Service*)

(*Received for publication August 26th*, 1938)

Classically, as its name indicates, arcus senilis has been associated with senility, although an identical appearance occurs in the young and is even referred to as arcus juvenilis. The condition is conventionally ascribed to fatty degeneration of the corneal cells and fibrillae, and Schieck (1931) parallels the condition with that of atheromatosis in the vascular system, concluding that it is not an actual disease, but rather a change due to age, that it is generally harmless, but that, when very marked, it brings with it a danger of tissue-degeneration and necrosis of the peripheral parts of the cornea.

Eck and Désbordes (1935) found that the serum-cholesterol tended to increase with age. Kolen (1931) produced a comparable condition in rabbits by feeding them on ' cholesterol oil.' This arcus lipoides, however, was cleared by new vessel formation, whereas such formation had not apparently been described in human arcus senilis. He reported a case of a man of 48, suffering from alcoholism, myocardial degeneration and arteriosclerosis, whose blood-cholesterol was at the upper normal limit and who had an arcus senilis with new vessel formation. Strömgren (1932) noted in a Danish asylum that the condition appears in subjects of the pyknic type at an earlier age than in others—thus implying a relationship with hypercholesterolaemia. White (1935), working with material in the U.S.A., showed conclusively that arcus senilis should not be considered an essential sign of senility, as it does not occur in a sufficiently high percentage of those reaching old age ; nor should it be considered an essential sign of arteriosclerosis, nephritis or hypertension, because of the low percentage of its occurrence in cases showing these conditions. There is no doubt, however, that arcus senilis is often notably associated with a hyper-cholesterolaemia.

It seems also that it may be associated with a hypocholesterolaemia, for Mulock Houwer (1934) reports that it is very frequently found in Javanese, and they, he notes, have a low blood-cholesterol. Alpago-Novello (1894) is quoted by Harris (1919) as having observed the condition frequently in Italian pellagrins. During a dietetic survey in Kordofan in 1934, in the course of examining 11 male Nubas, the present writer noted that 4 of them (36 per cent.) showed arcus senilis ; their ages averaged 44. This year, at Sennar in the Blue Nile Province, the writer examined for this sign 60 hospital-patients and 67 inmates of the local prison, selecting for examination all below the age of 40. The sign was

found in 13 per cent. and 10 per cent. respectively. Relevant Sudanese diets are composed mainly of millet, and are thus deficient in protein. Half the year is a period of desiccation, and the result is a deficiency more or less chronic in vitamins A, B_2 and C—most notably, perhaps, in C. It is safe to assume a dietary of comparable quality for Italian pellagrins and also for the rice-eating Javanese. A further factor common to these communities of lower latitudes, as contrasted with those of more northerly latitudes, is brighter sunlight. This implies increased ultra-violet irradiation, a factor which accentuates or accelerates the signs and symptoms of vitamin deficiencies. It is of interest to note that Roffo (1935) found that the effect of irradiation on the cholesterol-content of the skin of rats confirmed his opinion that the actual amount of cholesterol in the skin is increased by exposure to sunlight or ultra-violet light.

Corkill (1934), investigating pellagra in Sudanese, was impressed with the frequency of the sebaceous dysfunction on the face—an appearance known in the literature as ' sulphur-flaking.' He was led to believe that a marked disturbance of the cholesterol metabolism was involved, particularly as the dietary was deficient in sterols during the dry season. Unfortunately, arcus senilis was neither noted nor sought for. The fact that not all Sudanese, nor yet even all tribal Sudanese, have arcus senilis suggests that diet rather than sunlight is more likely to be the primary factor of the two ; and, on the whole, it seems a reasonable working hypothesis to say that arcus senilis is associated with a cholesterol dysfunction which may be causally related to a food deficiency, such deficiency being capable of accentuation or acceleration by exposure to bright sunlight. It seems that this deficiency may be of protein and/or of vitamins A, B_2 and C, either jointly or individually. It may be noted here that Gander and Niederberger (1936), studying hospital-patients over 50 years of age, found a characterististic lack of vitamin C, and, amongst associated conditions of disease, attributed arteriosclerosis to this deficiency. The second of the two cases now to be discussed appears to show that deficiency of this vitamin may be of importance in the causation of arcus senilis.

The two Sudanese military officers shown in Plate VIII are from the same unit, and both exhibit, among other eye-signs, well-defined arcus senilis. Case A came for consultation because he was worried about the astonishingly sudden appearance of the sign ; and Case B, his colleague, came as a matter of interest, because he had developed the same condition himself at the age of 27. Case A developed his condition in 1934, at the age of 32 ; the history is noteworthy. On January 3rd, 1934, in the early dry season, he left Kordofan with a motor-patrol for the Libyan Desert. He was emphatic that he had no arcus senilis when he started. At the beginning of April, i.e., three months later, he noticed the appearance in a mirror. The patrol returned on July 13th, 1934, about five weeks after the opening of the rainy season. He was seen and photographed by the writer on November 2nd, 1934.

He complained of nothing but the arcus senilis, had enjoyed good health while on patrol, and had not suffered from night-blindness. On examination it was noted that his tongue was pink and healthy, as were also his gums and buccal mucosa, except that on the latter was an impression of his closed teeth, as sharply defined as those noted as being characteristic of the severer cases of pellagra examined by Corkill (1934). Gingivitis, dental caries and other signs and symptoms were absent, except for the yellow-brown pigmentation of the conjunctiva (apparently precursory to pterygium and pinguecula) which is common in Sudanese.

He was strong, dark-skinned, of good general nutritional status, robust, active, and of good class, i.e., his living conditions would normally be of relatively high standard. On patrol, the diet had consisted mainly of dried lentils, wheat biscuits, tinned apples and pine-apples, tea, sugar and salt. The diet was supplemented from time to time by fresh meat, clarified butter, fresh tomatoes and fresh green-leaf vegetables, taken out by special convoy. No milk was ever procurable. The routine diet was thus deficient in protein and in vitamins A and C—particularly in vitamin C, for the lentils and tinned fruit may be considered to have contributed a fair amount of protein and of vitamins A and B_2.

Scurvy was probably not experienced in its classical form because of the short period of more-or-less absolute deprivation. The deficiency of vitamin C really amounted to an accentuation of what is almost certainly the commonest food deficiency in the Sudan, that is, a chronic suboptimal vitamin C intake. The absence of comments on arcus senilis in the literature of scurvy may be due to the fact that, whereas classical scurvy has usually occurred after six months or so of acute and absolute deprivation, arcus senilis in tropical communities appears to develop slowly in those on a chronic, but not absolute, vitamin C subnutrition. The case discussed above may, therefore, have had the formation of his arcus senilis very much accelerated by acute absolute deprivation, coupled with exposure to fierce insolation, and may also have been on the verge of florid scurvy.

It seems, then, that arcus senilis may be a sign of chronic vitamin C deficiency ; and, further, it seems that the impression of the teeth on the insides of the cheeks, especially when heavily defined, may also be a sign of such deficiency, for not only did the case discussed above show this appearance markedly, but Corkill (1934) found it a characteristic of the severer cases in a community of Sudanese pellagrins whose diet lacked, amongst other food principles, vitamin C.

The question of why all vitamin C deficient persons do not show an arcus senilis opens up a subject beyond the scope of this note, but two rather obvious explanations offer themselves at once. Some will ingest more of the vitamin than others, and—what is more important—there are differences between individuals which depend on factors intrinsic to the body and which affect digestion, absorption and utilization of the vitamin.

SUMMARY AND CONCLUSIONS

1. Arcus senilis, though found in various conditions associated with hypercholesterolaemia, is not an essential sign of such conditions.

2. It occurs frequently in Javanese who have a hypocholesterolaemia.

3. It occurs frequently in Italian pellagrins and in Sudanese whose diets are low in protein and in vitamins A, B_2 and C.

4. Common to the Javanese, Italian and Sudanese communities are the climatic factors of high temperature and bright sunlight. Such factors accentuate vitamin deficiency.

5. A young Sudanese exposed to a severe desert environment, on a diet particularly lacking in vitamin C, developed an arcus senilis, but not scurvy, in three months.

6. It is suggested that arcus senilis may be a sign of chronic vitamin C subnutrition.

7. It is also suggested that the impression of the teeth on the inside of the cheeks, when markedly defined, may be a further sign of chronic vitamin C subnutrition.

8. If the writer's hypothesis is correct, vitamin C subnutrition is related to cholesterol metabolism.

ACKNOWLEDGEMENTS.—The writer is indebted to Dr. E. D. Pridie, Director of the Sudan Medical Service, for permission to publish this paper, and to Dr. F. L. Wheaton, for drawing the writer's attention to the case described.

REFERENCES

ALPAGO-NOVELLO (1894). Osservazioni antropologico-cliniche sui pellagrosi. *Riv. Venet. Sci. Med.*, **20**, 529. (Quoted by Harris, H. F. (1919): Pellagra. p. 220. New York.)

CORKILL, N. L. (1934). Pellagra in the Sudan. *Jl. Trop. Med. & Hyg.*, **37**, 197, 216-18.

ECK, M., and DÉSBORDES, J. (1935). Influence de l'âge sur les variations de la cholestérinémie et du pouvoir cholestérolytique. *C. R. Soc. Biol.*, **118**, 498.

GANDER, J., and NIEDERBERGER, W. (1936). Ueber den Vitamin C-Bedarf alter Leute. *Münch. Med. Woch.*, **83**, 1386.

KOLEN, A. (1931). Über einen Fall von ungewöhnlichem Arcus senilis Corneae mit oberflächlicher Blutgefässneubildung. *Klin. Mbl. Augenheilk.*, **86**, 204. (Abstract in *Zentralbl. f. Ges. Ophthal.*, **25**, 415.)

MULOCK HOUWER, A. W. (1934). Arcus senilis und Pterygium. *Ned. Tijdschr. Geneesk.*, **78**, 2456. (Abstract in *Zentralbl. f. Ges. Ophthal.*, **32**, 357.)

ROFFO, A. H. (1935). Action des rayons solaires (ultraviolets) sur la peau, et accumulation de cholestérine. *Bol. Inst. Med. Exp. Cáncer, B. Aires*, **12**, 583.

SCHIECK, F. (1931). Die Erkrankungen der Conjunctiva, Cornea und Sclera. (Schieck and Brückner: Kurzes Handbuch der Ophthalmologie, **5**, 377.)

STRÖMGREN, E. (1932). Konstitutionstypus und Arcus senilis. *Acta Psychiat., Kbh.*, **7**, 823. (Abstract in *Zentralbl. f. Ges. Ophthal.*, **28**, 189.)

WHITE, C. E. (1935). The relation of arcus senilis to arteriosclerosis and senility. *New Engl. Jl. Med.*, **212**, 13.

PLATE VIII

EXPLANATION OF PLATE VIII

Fig. 1. Case A, aged 32, in whom arcus senilis developed in three months during military service in the Libyan Desert. His diet at the time was lacking in vitamin C.

Fig. 2. Case B, showing arcus senilis which developed at the age of 27.

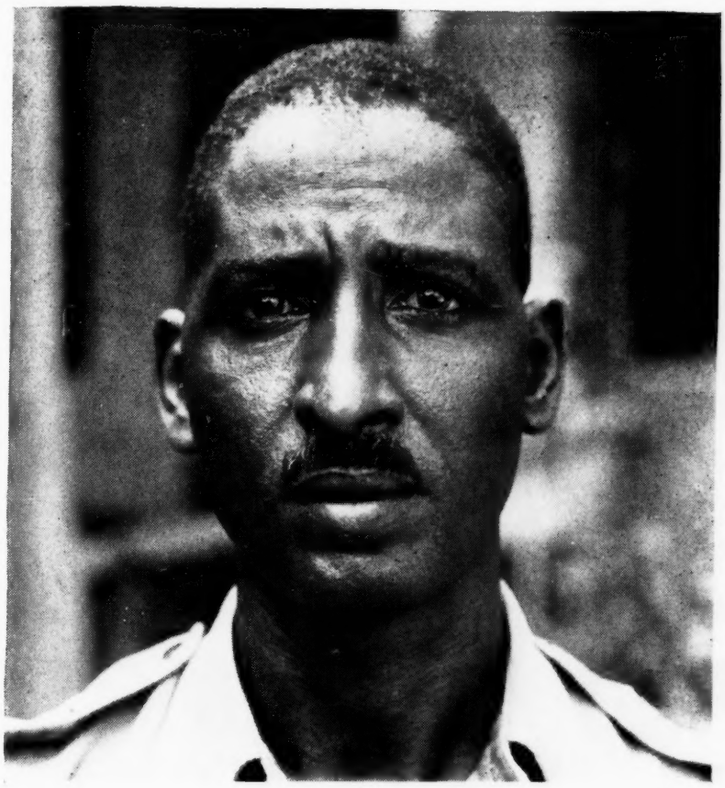


Fig. 1

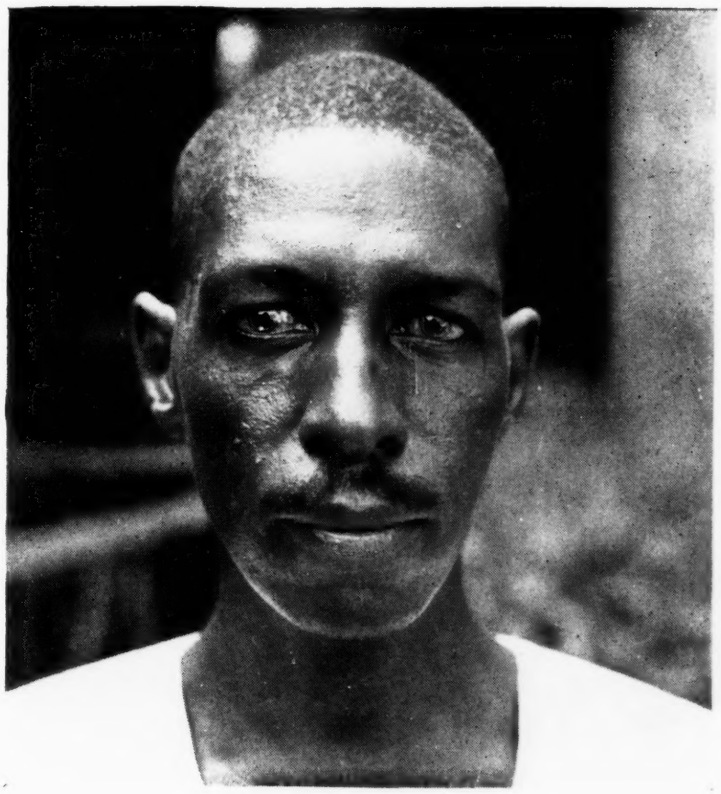


Fig. 2

H. R. Grubb, Ltd., Poplar Walk, Croydon

UNIV. OF MICH.

A LABORATORY STUDY OF ABYSSINIAN LOUSE-BORNE RELAPSING FEVER

BY

R. KIRK*

(*From the Stack Medical Research Laboratories, Khartoum, Anglo-Egyptian Sudan*)

(*Received for publication September 17th*, 1938)

CONTENTS

		PAGE
I.	INTRODUCTION	339
II.	MORPHOLOGY AND MOVEMENTS OF THE SPIROCHAETES ...	340
III.	CULTURE	341
IV.	ANIMAL INOCULATION	343
V.	TRANSMISSION	346
VI.	IMMUNITY	348
VII.	EFFECT OF SPLENECTOMY	349
VIII.	RESIDUAL INFECTIONS	352
IX.	SUMMARY	353
X.	REFERENCES	353

I. INTRODUCTION

During the past two years, cases of relapsing fever have been recorded in the Anglo-Egyptian Sudan, and the writer has had the opportunity of studying some of these. The complete restriction of the disease to immigrants from Abyssinia and Eritrea leaves no doubt that these countries are the source of the infection. The tendency to extension at the present time is probably related to the recent military upheaval in East Africa, for relapsing fever is essentially one of those diseases which flourish in times of war.

Relapsing fever of Abyssinian origin has not previously been encountered in the Sudan, although the existence of the disease in Abyssinia has been known for many years. The infection was first reported by Doreau (1908), and in the same year Brumpt (1908) reported that he had successfully infected a monkey in Paris by means of *Ornithodorus moubata* sent to him by a missionary from Harrar, thus establishing the existence of a tick-borne strain. Mesnil (1908), in a review, called the spirochaete *Sp. abyssin*; and Bergsma (1928) gave an account of some clinical cases, and noted the prevalence of *Ornithodorus moubata* and *Ornithodorus savignyi*.

Louse-borne relapsing fever has been reported from Addis Ababa by Sibilia (1937). This author claims to be the first to report the louse-borne disease; but Nägelsbach (1934), three years earlier, had noted that in the highlands of Abyssinia, where ticks were not found, lice and relapsing fever were both common, and concluded that the lice must be the vectors.

---

*The writer is indebted to the Director of the Sudan Medical Service for permission to publish this article.

It is interesting to note that de Paoli (1930), describing an epidemic of relapsing fever in Asmara in 1929, in which ticks were not found but lice were plentiful, states that the disease was imported into Eritrea from Abyssinia.

Corroboration of his views is given by Bruns (1937), who records a very fatal epidemic of louse-borne relapsing fever in the Djig district of Abyssinia in 1929. According to Cacciapuoti (1936), the disease is now endemic in many towns and villages in Eritrea.

According to Bruns (1937), relapsing fever is endemic in many districts of Abyssinia, but is apt to break out in epidemic form during the rainy season. The louse, *Ornithodorus moubata* and *Ornithodorus savignyi* are all cited as the vectors by this author.

It may be noted that, with the exception of Brumpt (1908), these authors rely largely on epidemiological data for their views on transmission, and do not substantiate them by experimental transmission of the disease by the alleged vectors.

The writer has carried out a laboratory study of the spirochaetes from some of the cases seen in the Sudan. It has been possible to incriminate the louse as the vector, and the results of animal inoculation have been concordant with eleven strains obtained from the blood of infected patients, and one strain isolated from an infected louse.

II. MORPHOLOGY AND MOVEMENTS OF THE SPIROCHAETES

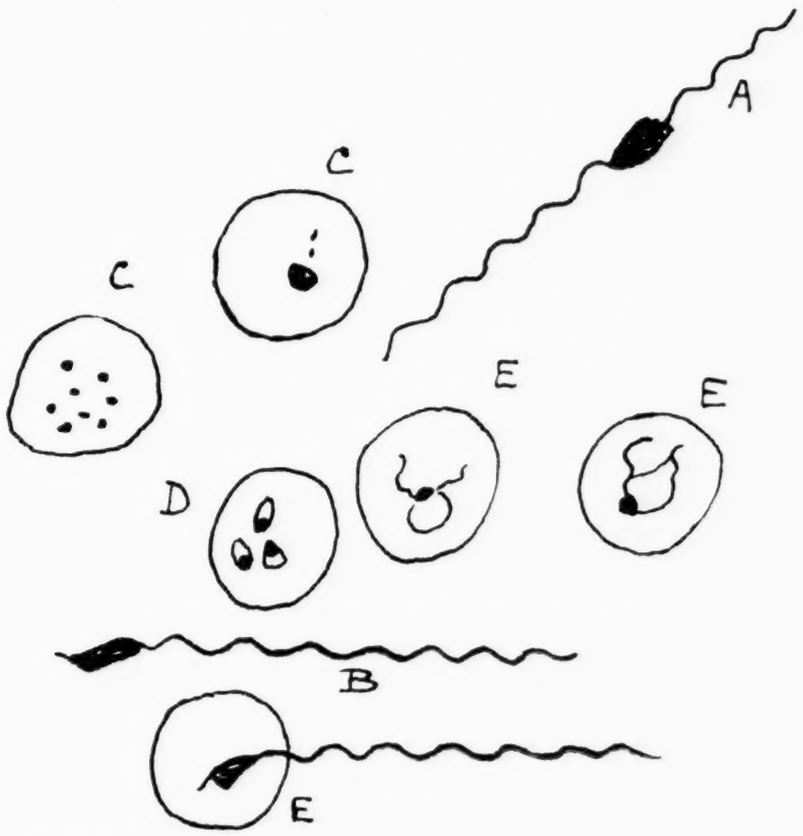
Typical spirochaetes (*Borrelia*) were found in the blood of the patients during the pyrexial stages of the disease. In stained films and under the dark field, their morphology and movements correspond closely with text-book descriptions and need not be detailed here.

Variations in length and in the number of turns in the spiral were considerable. It was found that by freezing citrated blood containing spirochaetes, keeping it for a few days in the ice-chest, and thereafter making films, beautiful preparations could be obtained, in which the regular spiral structure of the organisms was preserved (Plate IX, fig. 2). These films were ideal for measurements. The average length of 200 consecutive spirochaetes seen in such a film was found to be 18μ, the extremes being 10μ and 40μ. Wider extremes were observed in other films.

In these films of frozen blood irregular globose swellings were frequently seen in the spirochaetes, sometimes terminal, sometimes situated at various distances along the length of the spirochate. They probably correspond to the swellings seen by Butler (1908) in *Sp. duttoni* which had been kept for some time outside the human body. In all probability they represent degenerative changes.

In these films of frozen blood, too, various structures could be seen inside the red blood corpuscles. Some of them recalled the appearances figured by Balfour (1911) in the development of the intracorpuscular granules of *Sp. anserina*

(*Spirochaeta granulosa penetrans*) (see text-fig.). Indeed, all the appearances seen by Carter (1908) could be readily identified in the corpuscles of these films. Carter regarded them as stages in the life-cycle of the spirochaete. The appearances seen in the frozen blood certainly suggest penetration of the red



TEXT-FIG. Doubtful appearances in films of frozen blood. Composite diagram drawn under microprojector (×2,500 approx.). A.—Swelling in the middle of a spirochaete ; B.—Terminal swelling in a spirochaete ; C.—Granules in red blood cells ; D.—Babesia-like appearances in red blood cells ; E.—Appearances suggesting penetration of red cells by spirochaetes and disintegration of the spirochaetes inside the red cells.

blood cells by the spirochaetes, but it is more reasonable to regard them as artefacts, because :

1. They were seen only in frozen blood, never in fresh films.
2. The spirochaetes showing these peculiarities were always motionless in the dark field, and therefore presumably dead.
3. Other and obvious artefacts were observed in the same films.

III. CULTURE

Yuan-Po's medium. It was found impossible to grow the spirochaetes in the egg-medium described by Yuan-Po (1933, 1936). At the outset much time was lost by confining one's attempts at culture to this medium, and refusing to accept repeated failures, largely owing to the glowing reports of Yuan-Po,

who claimed to have obtained 100 per cent. successes with *T. recurrentis* in this medium.

Spirochaetes could be found in the cultures for periods up to 10, and even 20, days after inoculation. From the seventh day onwards their movements under the dark field became very feeble. Finally they stopped, and the spirochaetes disappeared. It was not possible to determine the manner in which they disappeared. Short, immobile forms of only two or three turns were observed, and probably resulted from the disintegration of dead or dying spirochaetes.

Batches of medium were made up according to the original recipe (Yuan-Po, 1933), as well as according to the modified simple method (Yuan-Po, 1936); but there was no difference in the result. No evidence of multiplication could be adduced in either medium. The instructions of the author were followed to the letter in both cases, and the resulting pH was found to be identical with that obtained by him, so that there is no reason to believe that the media differed in any way from those used by Yuan-Po.

After many attempts this medium was finally discarded, and other methods were tried.

Citrated human blood, kept either at room-temperatures or at 37° C., was not found successful. The spirochaetes died rapidly, as estimated by their movements under dark-ground illumination. They were always completely immobile in three days, and there was no evidence of multiplication. It was found that, as the corpuscles sedimented, the spirochaetes tended to settle in the layer just above the corpuscles, and a false impression of multiplication might be obtained if fluid from this layer were selected for examination. In some cases a tendency to auto-agglutination was observed. The blood used was of human origin, taken from the infected patients and containing spirochaetes at the time of withdrawal.

Diluted human serum, far from being a successful culture fluid, appeared to have a lethal action on the organisms, which were all motionless in 24 hours.

Diluted horse serum (Kligler and Robertson, 1922) was similarly found to be useless as a culture medium. Although six tubes were inoculated from two different strains, it should be admitted that the serum was all obtained from one horse; Hoeltzer and Zabolotzkaja (1926) have stated that sera from different horses often vary considerably, one giving a good growth, while another appears to have an inhibitory effect.

Excellent cultures were finally obtained in a *modified Noguchi's medium*, prepared as follows:

Under aseptic precautions, half an inch of egg albumen was pipetted into the bottom of 5 in. $\times \frac{1}{4}$ in. test-tubes, and then coagulated by placing the tubes in hot water at approximately 80° C. Thereafter the tubes were filled to within an inch and a half from the top with freshly drawn ascitic fluid. The tubes were inoculated by introducing 3–5 drops of blood containing spirochaetes, and

incubated at 37° C. Within 24 hours there was an obvious increase in the ease with which spirochaetes could be found in the dark field, and the high proportion of long and dividing forms was evidence of active multiplication. On the third day after inoculation, each dark field from the fluid lying just above the coagulated egg-white contained 20–30 actively motile spirochaetes, with a high proportion of dividing forms. Enormously long filamentous forms were sometimes encountered. In one of these the appearance of thinned parts along its length suggested that it was in the process of dividing transversely into four daughters.

This medium never failed to grow the spirochaetes. The impression was gained that growth was improved by the addition of 5–10 drops of sterile 50 per cent. glucose, but it was difficult to be certain. The addition of the glucose was certainly not necessary in order to obtain a good growth.

The addition of a layer of liquid paraffin on the top of the ascitic fluid was found to have no influence on the growth. Culture tubes without the paraffin on the top were indeed preferable, as the oil tends to adhere to the pipette with which samples are withdrawn for examination, and refractile oily globules appear in the dark field. As compared with Yuan-Po's medium, which is full of granular débris, the ascitic fluid gives beautifully clear dark fields, against which the spirochaetes stand out distinctly.

Subcultures were readily made by introducing 3–4 drops from a culture showing plentiful spirochaetes into a fresh culture tube by means of a capillary pipette, adding at the same time 3–4 drops of citrated or freshly drawn rabbit's blood. One strain was passed through 12 successive subcultures at 2–4 day intervals without any apparent loss of vitality, and the process was only given up because of the writer's departure on leave. After nine subcultures, the inoculation of $\frac{1}{2}$ c.cm. of the culture fluid into the peritoneum of a mouse resulted in the appearance of spirochaetes in the peripheral blood in 24 hours. The course of this infection was not followed out.

IV. ANIMAL INOCULATION

Rodents

Of laboratory animals, white rats, rabbits and guinea-pigs proved insusceptible. So also did the ordinary brown and black rats (*Rattus rattus* and *Rattus norvegicus*) caught locally by the Khartoum Sanitary Service.

This, at the outset, suggests a louse-borne infection. Nicolle (1932) recommends guinea-pig inoculation as the most ready method of differentiating between louse-borne and tick-borne strains in northern Africa, and suggests that, if this procedure were resorted to more frequently, many infections in Egypt, Algeria and Libya at present regarded as louse-borne would prove by their virulence to guinea-pigs to be due in reality to tick-borne strains.

White mice, obtained originally from the Wellcome Bureau of Scientific Research, London, were found to be susceptible. The infection in these animals was observed to run a very constant course. As a general rule the intraperitoneal

route was used for inoculating the spirochaetes. Spirochaetes were invariably found in the peripheral blood 12–24 hours after inoculation. They were always present 48 hours after inoculation, but usually disappeared during the following 12 hours. The infection ran an exceedingly mild course in these white mice. It was apparently symptomless, and daily examination of the blood for 20 days after the disappearance of the spirochaetes failed in every case to show any evidence of relapse. Subinoculations from mouse to mouse were not successful, even when a large inoculum of infected material was given. This was effected by bleeding a mouse to death into sterile citrate solution 30 hours after inoculation, when the blood, as shown by films taken immediately before bleeding, contained numerous spirochaetes. About three-quarters of a c.cm. of blood was obtained in this way, and inoculated immediately into the peritoneum of two fresh mice, but no infection resulted. Negative results were likewise obtained when the organisms were inoculated subcutaneously instead of intraperitoneally, and also by the percutaneous method, which Tomioka (1924) had found to give rise to a much more severe infection with the European strain in mice than intraperitoneal inoculation.

According to the tables drawn up by Balfour (1911) and Manson-Bahr (1935), passage through monkeys increases the virulence of the European strain of *T. recurrentis* for small rodents. This was not found to be the case with the Abyssinian strain. Rats, rabbits and guinea-pigs were still refractory after passage of the spirochaetes through monkeys, and the virulence of the organisms for white mice was, if anything, diminished. Twenty-four hours after inoculation the spirochaetes were present in the peripheral blood of the mouse, but at 48 hours they were exceedingly scanty and difficult to find. After this they disappeared and no relapse took place. Subinoculations from mouse to mouse were still unsuccessful.

It is interesting to note that the course of the infection in these mice inoculated from monkeys did not vary with the severity of the disease in the monkeys. Nor did it seem to signify whether the original monkey was splenectomized or not, or whether it was originally infected by the inoculation of blood from a case, or by experimental transmission from an infected louse. These differences made a considerable difference in the severity of the infection in the monkeys.

The gerbille, or desert rat of the northern Sudan, was also susceptible, and in this animal the course of the infection was similar to that in mice, but the parasites sometimes persisted in the blood for three or even four days. Subinoculations from gerbille to gerbille sometimes resulted in a transitory appearance of scanty spirochaetes about 48 hours after inoculation. The gerbille seemed undoubtedly to be the most susceptible of the available small rodents.

An interesting observation made in these rodent infections was that in a small percentage of white mice the spirochaetal infection was accompanied by, or more commonly followed by, a low-grade septicaemia in which a mixture of

organisms were concerned, principally diphtheroids, cocci and bacilli. This always persisted for some time after the spirochaetes had disappeared from the blood, and generally seemed not to upset the health of the animals, although in two mice the number of organisms observed in the blood-films steadily increased, and the animals became ill, and finally succumbed. Post-mortem examination revealed no peritonitis or abscess-formation, and clinical examination of other affected mice showed no evidence of peritonitis or local reaction at the site of inoculation.

At the time, not much attention was paid to these organisms which were seen in the blood-films, except to exclude the possibility of their being due to faulty technique at the time of taking the blood-films. It is difficult to understand why the intraperitoneal inoculation of spirochaetes withdrawn directly from a human vein should cause a septicaemia without local reaction, unless the organisms were of extreme virulence, in which case the septicaemia might reasonably be expected to be rapidly fatal.

It is possible that the condition may have been due to an increase in the invasive powers of commensual organisms, brought about by the spirochaetal infection. Sanarelli (1927) maintains that the spirochaetal infection is harmless in itself, but in some way is able to increase the invasive powers of other organisms, and that these are responsible for the disturbance which takes place. Kulescha and Titowa (1923) stated that many of the relapsing fever fatalities in the Russian epidemic of 1920–1921 were due to secondary infection, and pointed out that from the literature it is apparent that many of the past European epidemics have been complicated by a pyaemia in the same way.

Monkeys

The common grey monkey of the Sudan (*Cercopithecus sebaeus*) was found to be susceptible, and in these animals the infection is similar to the human disease. The spirochaetes commonly appear in the peripheral blood after an incubation period of 2–3 days, persist for 2–5 days, and then, after a lapse of some 10 days, reappear for 1–3 days, after which there is no further relapse. Some variation between individual monkeys was encountered. Thus, one monkey had no relapse, and another was found which appeared to be quite immune : attempts to infect it with two different strains were quite unsuccessful.

It was found that one monkey could readily be infected by the inoculation of blood containing spirochaetes from another monkey. A strain obtained from infected human blood was passed through two monkeys in this way, and one obtained directly from a louse was passed through three successive monkeys. No attempt was made to ascertain how many passages could be effected in this way without the strain losing its virulence. It may be recalled that Sergent and Foley (1910) were unable to pass the Algerian strain even to a second monkey, and that Gambier (1923) in the French Sudan had the greatest difficulty in infecting even one monkey.

Enlargement of the liver and spleen was readily detected in all the infected monkeys, but no attempt was made to follow the clinical course of the disease in these animals. Except for very obvious illness, attention was confined to the examination of the blood for organisms.

In general terms the virulence of the strain for laboratory animals may be summarized as follows. Rodents, with the exception of white mice and gerbilles, were insusceptible. In these two latter animals the infection runs an exceedingly mild course, ending in recovery. There is no relapse, and the strain cannot be maintained in these animals. In monkeys the infection runs a mild clinical course with one relapse, and tends to recovery. Passage through at least three monkeys is possible.

These results conform in the main with those which have been found fairly universally with the louse-borne strains of relapsing fever, and especially with the north African group.

V. TRANSMISSION

Spirochaetes found in lice. It has been possible to demonstrate that the louse is the intermediate host of the relapsing fever under study. Metacyclic forms of the spirochaete were found in the haemocoele fluid of lice from infected persons. Compared with the organisms found in the blood, these forms are smaller, finer, and more regularly spiral in structure. Stained by Leishman's method they do not show the same tendency to twist themselves into figures-of-eight and other coiled or irregular forms. They were frequently found in enormous numbers, forming large tangled masses in the haemocoele of the louse (Plate IX, fig. 4).

The best method of demonstrating the spirochaetes in the louse was found to be that described by Riding and Macdowell (1927). The louse was seized with fine forceps, and laid on its back in a small drop of distilled water on a clean slide. The abdomen was transfixed by a needle, lateral to the gut, and the haemocoele fluid was allowed to escape from the puncture, the movements of the legs of the louse assisting the mixing of the haemocoele fluid and the water on the slide. The fluid could then be examined by dark-ground illumination or allowed to dry in the air and stained by Leishman. This method was infinitely superior to making smears of crushed lice, and gave beautiful preparations, free from débris. The lice were kept in a test-tube for 24 hours between removal from the patient and examination for spirochaetes.

The percentage of infected lice varied in different cases, presumably according to the duration of the disease, although it was not possible to verify this, as histories were unreliable, and steps were taken to cut short the disease with treatment as soon as the diagnosis was verified under the microscope. Most commonly 15–20 per cent. of the lice were found infected. In 30 per cent. of cases no infected lice were found. In one case the infection rate was as high as 60 per cent. (6 infected out of a total of 10 lice obtained from the patient's clothing).

No infected nits found. Both male and female lice were found to be infected. No evidence of hereditary transmission in the louse was found, although large numbers of nits were examined, including a number actually deposited in captivity by infected lice. Microscopical examination alone was made : the nits were crushed on a slide and examined under the high power stained by Leishman. No animal inoculation experiments were carried out, so that the possibility of hereditary infection by an invisible granule stage was not investigated.

Transmission of the disease to a monkey from a louse. The spirochaetes in the lice were motile under dark-ground illumination, and they were able to reproduce the disease in monkeys. Only one successful transmission experiment was carried out, but this took place under somewhat dramatic circumstances.

A blood-slide from a patient admitted to the Church Missionary Hospital in Omdurman on February 24th, 1938, was found to be positive for relapsing fever. The hospital was informed, and it was requested that the lice (if any) from this patient be collected and transmitted to the writer. Unfortunately, it transpired that the patient had already been washed and his clothes removed for disinfestation.

On the following day, however—thanks to the Medical Officer of Health, Khartoum—a single louse was found on a pair of pants, which must somehow have escaped the delousing process. This louse was given a feed from the writer's arm, and kept for another day at 37° C.

On February 26th, 1938, this louse was transfixed on a slide and its haemocoele fluid was allowed to mix with distilled water in the manner described above. Some of this fluid was rubbed into a small scarified area on the shaved abdomen of a splenectomized monkey, and allowed to dry, as in a vaccination. The residue on the slide was stained by Leishman's method, and examined microscopically. It was found to contain spirochaetes.

After an incubation period of four days spirochaetes appeared in the monkey's blood. During the next eight days the spirochaetes became progressively more numerous, until there appeared to be as many organisms as red blood cells. The monkey became obviously ill on March 6th, 1938, and died on March 10th, eight days after the first appearance of the spirochaetes in the blood.

According to this observation, the disease acquired from the louse is more virulent than that which results from the inoculation of blood containing spirochaetes. In no other experimental animals were the spirochaetes observed in the peripheral blood in such enormous numbers as in this monkey, which was, moreover, the only animal to die in the first paroxysm of experimental relapsing fever. Although this is in accordance with the experimental findings in tick-borne relapsing fever, Lipstein (1936), working with *S. novyi*, found that the infections derived from lice were always benign, while those which followed the inoculation of infected blood were always fatal.

Two similar attempts had previously been made to infect this monkey by means of lice 30 and 35 days earlier, but both were unsuccessful. In both cases, however, examination of stained smears of the haemocoele fluid of the lice showed that the latter were not infected.

It was considered that these experiments, together with the results of animal inoculation and the demonstration of infected lice in a large proportion of the human cases, were sufficient to incriminate the louse as the vector, and no further experiments were carried out along these lines.

VI. IMMUNITY

Evidence of immunity. On January 24th, 1938, blood containing numerous spirochaetes was inoculated from a human case in Khartoum North directly into two mice which had previously been infected on two occasions, and at the same time into two fresh mice. In 24 hours the latter developed a typical 2-days' infection, while spirochaetes failed to appear in the blood of the two previously inoculated mice. A similar result was obtained on February 26th, when blood was inoculated from an infected monkey into two fresh mice and two previously inoculated mice.

A monkey (no. R1) proved resistant the second time an attempt was made to infect it. Another monkey (no. R2), which had been twice infected previously, was insusceptible when a third attempt was made on March 5th. On both these occasions it was possible to infect other monkeys by inoculation of the same blood which failed to infect monkeys no. R1 and R2 respectively, showing that the absence of infection was not due to loss of virulence of the spirochaetes.

Evidence exists, therefore, that a state of immunity to further infection can be brought about by a previous infection. But this is inconstant and erratic. Other and more numerous experiments can be cited in which previous infection failed to protect against further infection. Thus, two mice infected on November 13th, 1937, were found to be susceptible again on November 16th, and two mice infected on November 16th were as readily susceptible again on November 25th. In both cases the second infection was similar to the first in its intensity and duration. The same is true with monkeys: monkey R1 was susceptible on one occasion and thereafter resistant, while monkeys R2 and R3 were readily infected a second time.

Presence of several antigenic variants. These discrepancies are probably due to antigenic differences in the various strains studied. Baltazard (1936) and other workers have found that no two strains of relapsing fever are the same, and that infection with one strain results in the development of immunity against that strain only and no other.

Cunningham (1925) has postulated the alternation of two serological variants with successive relapses. Some of our experimental results at least suggest that the ' relapse ' strain is different from that of the first paroxysm.

On January 24th, 1938, two monkeys and two mice, all of which had previously been infected and had recovered, were inoculated with blood containing numerous spirochaetes from a case in Khartoum North. Of those four animals, one of the monkeys (no. R2) was the only one afterwards to show spirochaetes in its blood, although they had all been previously infected with one strain. On this previous occasion, however, one of the monkeys (no. R1) relapsed, while the other did not (no. R2). The mice were both inoculated from monkey no. R1 during the first paroxysm and again during the relapse. On both these occasions they were susceptible. The fact that both mice were resistant on the third inoculation, as well as monkey no. R1, suggests that the difference between the two monkeys on this occasion was due to other factors than individual variations in natural resistance.

It is reasonable to assume that the resistance of the one monkey and the two mice was due to acquired immunity brought about by previous infection with an antigenically similar strain. That the immunity was not due to the first paroxysm is shown by the successful infection of the other monkey (no. R2), which also had a first paroxysm in the previous infection. Presumably, therefore, the immunity in monkey no. R1 and the two mice was due to the relapse, which never appeared in monkey no. R2. If this be so, then the original and relapse strains were probably different from each other.

Another monkey (no. R3) had a relapse during its first infection, and thus probably experienced two different variants. This did not prevent its being successfully infected two months later by the inoculation of infected blood from another monkey. It is probable, therefore, that more than two antigenic variants were present among the strains.

There is possibly also a factor in the immunity which is not specific to the strains. Two infections seemed always to make our animals immune to further infection. It may be noted, too, that the relapse is usually of much shorter duration than the original attack. It may further be observed that, although the number of our experiments on this point is small, when previously infected monkeys are splenectomized, and then inoculated with a strain to which they are susceptible, the effect of the splenectomy on the infection is different from that which results when splenectomy is done before the first infection.

VII. EFFECT OF SPLENECTOMY

According to Meleney (1927), Kritschewski and Rubinstein (1927) and Kalajew (1931), the increased susceptibility of experimental animals to relapsing fever which results from splenectomy is evidenced by a much heavier infection of the blood with spirochaetes and an increase in the death-rate of inoculated animals. On the other hand, Breinl and Kinghorn (1906) found that splenectomy had no effect whatever on the course of African relapsing fever, and Velu, Balozet and Zottner (1931) have reached a similar conclusion with regard to

T. hispanicum infections. Russell (1931) found with the West African strain that splenectomy might result in a slightly higher death-rate, but that otherwise it had no effect on the infection.

In experimental work with organisms of relatively low virulence, such as this Abyssinian strain, anything which will increase the susceptibility of the hosts, and make the infections a little more definite, is a practical advantage. The few experiments carried out by the writer under this heading were undertaken primarily to determine whether any advantage would be gained by the use of splenectomized rather than normal animals for transmission experiments, although, in point of fact, the experiments had already been undertaken with splenectomized animals.

Mice. Two mice, splenectomized five days previously, were inoculated intraperitoneally with approximately 0·2 c.cm. of infected blood withdrawn from a monkey on January 26th, 1938. Two normal mice were inoculated at the same time, as controls. In all four animals the disease pursued a similar course, spirochaetes being found in the blood during the first two days following inoculation, after which they disappeared, and did not return.

Six days after the disappearance of the spirochaetes, the two control mice were splenectomized, but this did not result in the reappearance of the spirochaetes.

Two of these four mice were infected a second time on March 5th, and the infection ran a typical course, exactly similar to that in two fresh mice inoculated at the same time. A third attempt to infect them 15 days later was not successful.

The effect of splenectomy on the course of the infection in mice appears to be negligible, although it is readily admitted that the number of these experiments is too small to allow of anything more than tentative conclusions being made.

Monkeys. In monkeys the results were different. The number of observations which we were able to make was restricted, but even these few indicated that splenectomy does modify the course of the disease in these animals, and does increase their susceptibility to the infection.

On March 5th, 1938, two splenectomized monkeys and one normal one were inoculated with blood containing abundant spirochaetes. In the normal monkey (no. R8) the infection ran a fairly typical course. In the splenectomized monkeys (nos. R7 and R6) no striking departure from the normal was noticed in the first paroxysm, but the relapses appeared earlier, lasted longer, and showed a much heavier infection of the blood with spirochaetes—almost comparable in this respect to the fatal infection of monkey no. R4, which was infected directly from the louse. In one of the splenectomized monkeys a second relapse was observed. The other subsequently died. Owing to the writer's departure on leave, it was not possible to follow out these infections fully, so that there may have been further relapses which were not observed.

It is perhaps hardly permissible to include in this comparison the monkey which was found immune, nor one which was infected directly from a louse, but their inclusion in the series gives a very striking picture of the effect of splenectomy, thus :

Character of disease	Five normal monkeys	Four splenectomized monkeys
Immune	1	—
No relapse	1	—
1 relapse	3	1
2 relapses	—	1
Death-rate	Nil	50 per cent.

One splenectomized monkey died during the first paroxysm, and another, which ultimately died, probably had more than one relapse.

Splenectomy had no effect on acquired immunity, whether undertaken before or during the second infection. When one normal and two splenectomized monkeys, all of which had been previously infected and had recovered, were inoculated a second time, the course of the second infection was no more severe in the splenectomized monkeys (nos. R1 and R2) than in the normal one (no. R3). Monkey no. R1 was, indeed, immune, and nos. R2 and R3 were susceptible. This was even more clearly demonstrated on March 5th, 1938, when a splenectomized monkey which had been infected twice previously was inoculated with the same blood as a normal monkey which had been infected once. The latter was susceptible, and the former was immune. Kritschewski and Rubinstein (1931) found that, while splenectomy had no effect on acquired immunity, it might cause the reappearance of the spirochaetes in the blood. This was not found in any of our animals.

A possible explanation why the effects of splenectomy were more apparent in monkeys than in mice is suggested by an experiment of Rubinstein (1930). This author found that with the ordinary doses of the infective material the death-rate was the same in splenectomized as in control rabbits. But when a much larger dose was used the death-rate of the splenectomized as compared with the normal animals was strikingly increased. The effect of the splenectomy was apparent only in an infection which was likely to run a severe course in the normal animal ; with very mild infections the reserves of the reticulo-endothelial system are sufficient to mask any effects of the splenectomy. In the case of our mice the course of the infection was very mild. But the more severe infection in the monkey entailed presumably a greater tax on the reticulo-endothelial system, which was then less able to withstand removal of a large part of its substance.

Rubinstein and Golubewa (1930) have shown that in *Trypanosoma equiperdum* infections the effect of splenectomy is inversely proportional to the length of time which elapses between the splenectomy and subsequent infection. In this connection it is interesting to observe that the only one of our monkeys in which the inoculation of infected blood resulted in a fatal issue was one which had been recently splenectomized. The others had undergone the operation several months previously, and in the meantime had received numerous inoculations of foreign material (tick contents), a process which might reasonably be expected to induce some degree of compensatory hypertrophy in the remainder of the reticulo-endothelial system.

VIII. RESIDUAL INFECTIONS

Wenyon (1926) and others have recorded that, when mice, previously inoculated with spirochaetes, were infected with trypanosomes several months after the apparent disappearance of the spirochaetes from the blood, this resulted in the reappearance of the spirochaetes in the blood-stream. Buschke and Kroó (1923) and other workers have shown that the spirochaetes, after their disappearance from the blood, may settle in the tissues of the central nervous system, where they may remain viable for long periods.

The importance of these observations from the point of view of the human infection is that they suggest the possibility of spirochaetes, lying dormant in the tissues of recovered patients, again invading the blood-stream as a result of some debilitating infection, e.g., malaria. This might lead to infection of lice, and start a new outbreak.

On January 13th, 1938, three mice, which had been infected with spirochaetes at different periods during the previous two months, were inoculated with *Trypanosoma evansi*. For this I am indebted to Dr. S. C. J. Bennett, Senior Veterinary Research Officer of the Sudan Government. After an incubation period of six days the trypanosomes appeared in the blood-stream. They persisted for four days only. In examinations of the blood carried out for the following 20 days, neither trypanosomes nor spirochaetes were seen.

On another occasion 12 white mice, which had been infected on one or more occasions at periods varying from 14 days to eight weeks previously, were killed. Emulsions were made of their brains, and inoculated subcutaneously into six gerbilles, each gerbille receiving material from two mouse brains. During the course of the following 12 days none of the gerbilles developed an infection, after which their blood was not examined.

Gerbilles were chosen because they had shown themselves to be the most susceptible of the small rodents to the infection, but the experiment was not hopefully undertaken. If passages were not successful when blood rich in spirochaetes was inoculated, they were hardly likely to succeed with brain emulsions containing possibly a few organisms. Negative results in this instance can have no significance.

Probably the only successful way to investigate this aspect of the Abyssinian strain would have been to use monkeys only. As the important point is not so much the proof of latent infection as to estimate the tendency of the strain to cause this, as shown by the proportion of ordinary infections which are followed by latent infection, any satisfactory work along these lines would have required far more monkeys than we had at our disposal.

IX. SUMMARY

1. An Abyssinian strain of louse-borne relapsing fever has been studied in the laboratory.

2. The organism has been successfully cultured in an egg-albumen ascitic fluid medium, but not in Yuan-Po's egg-medium or in other media.

3. Gerbilles and white mice were the only rodents found to be susceptible. In these animals the disease runs a very mild course. There is no relapse, and the strain cannot be maintained in these animals, even after passage through monkeys.

4. Monkeys (*Cercopithecus sebaeus*) were susceptible, and in them the infection runs a course similar to the human disease. It appears possible to maintain the strain in these animals for at least three passages.

5. Evidence of immunity was found in infected animals, but several antigenic variants of the spirochaete are apparently present.

6. In monkeys, splenectomy was found to influence the course of the first infection, but had no effect on acquired immunity. In mice, splenectomy had no effect at all. A possible explanation of these results is discussed.

7. No evidence of latent or residual infections was found in rodents after recovery.

8. The intermediate host is the louse. Virulent spirochaetes were found in lice from infected cases, which were able to reproduce the disease in a monkey.

9. In the monkey, the infection derived from the louse is more severe than that derived from the inoculation of infected blood.

10. This Abyssinian strain of relapsing fever resembles in its general features the other members of the north African group of louse-borne strains.

X. REFERENCES

BALFOUR, A. (1911). The spirochaete of Egyptian relapsing fever: is it a specific entity? *4th Rep. Wellcome Trop. Res. Lab.*, A, 74.

BALTAZARD, M. (1936). Individualité stricte des souches marocaines du spirochète hispano-africain. *Bull. Soc. Path. Exot.*, **29**, 667.

BERGSMA, S. (1928). Relapsing fever in Abyssinia. *Jl. Trop. Med. & Hyg.*, **31**, 289.

BREINL, A., and KINGHORN, A. (1906). An experimental study of the parasite of the African tick fever (*Spirochaeta duttoni*). *Mem. L'pool Sch. Trop. Med.*, **21**, 1.

BRUMPT, E. (1908). Existence de la 'fièvre des tiques' en Abyssinie. *Bull. Soc. Path. Exot.*, **1**, 432.

BRUNS, A. (1937). Ueber Rückfallfieber in Abessinien. *Arch. f. Schiffs- u. Trop.-Hyg.*, **41**, 343.

BUSCHKE, A., and KROÓ, H. (1923). Spinale Strangdegeneration nach experimenteller Rekurrens. *Deut. Med. Woch.*, **49**, 1435.

BUTLER, C. S. (1908). A peculiar characteristic of *Spirochaeta duttoni*. *U.S. Nav. Med. Bull.*, **2**, no. 4, p. 1. (Reviewed in *Jl. Trop. Med. & Hyg.*, **12**, 154.)

CACCIAPUOTI, R. (1936*a*). Febbre ricorrente nell'Acchelè Guzai (Eritrea). *Arch. Ital. Sci. Med. Colon.*, **17**, 289.

——— (1936*b*). Contributo allo studio della febbre ricorrente da pidocchi in Eritrea. *Ibid.*, **17**, 546.

CARTER, R. M. (1908). A preliminary note on spirochaetosis in southern Arabia, and the morphology of the parasite. *Ind. Med. Gaz.*, **43**, 370.

CUNNINGHAM, J. (1925). Serological observations on relapsing fever in Madras. *Trans. Roy. Soc. Trop. Med. & Hyg.*, **19**, 11.

DOREAU, P. (1908). Fièvre spirillaire en Abyssinie. *Bull. Soc. Path. Exot.*, **1**, 480.

GAMBIER, A. (1923). Essai de transmission aux animaux de laboratoire du spirochète de la fièvre récurrente soudanaise. *Ibid.*, **16**, 159.

HOELTZER, R., and ZABOLOTZKAJA, T. (1926). Vergleichendes Studium der Kultivierungsmethoden der *Spirochaeta obermeieri*. *Zentralbl. f. Bakt., Orig.*, **100**, 306.

KALAJEW, A. W. (1931). Ueber die Natur der Immunität bei Rückfallfieber, XIV. *Giorn. Batteriol. e Immunol.*, **7**, 184.

KLIGLER, I. J., and ROBERTSON, O. H. (1922). The cultivation and biological characteristics of *Spirochaeta obermeieri* (*recurrentis*). *Jl. Exp. Med.*, **35**, 303.

KRITSCHEWSKI, I. L., and RUBINSTEIN, P. L. (1927). Ueber die Natur der Immunität beim Rückfallfieber, I. *Ztschr. f. Immunitätsforsch.*, **51**, 27.

——— ——— (1931). Ueber die Natur der Immunität bei Rückfallfieber, XV. *Ibid.*, **71**, 372.

KULESCHA, G. S., and TITOWA, N. A. (1923). Die pathologische Anatomie und Aetiologie der Komplikationen des Rückfallfiebers. *Virchows Arch.*, **241**, 351.

LIPSTEIN, I. (1936). Transmission de *Spirochaeta novyi* par *Pediculus corporis*. *Ann. Parasitol.*, **14**, 113.

MANSON-BAHR, P. H., Ed. (1935). Manson's Tropical Diseases. 10th ed. London.

MELENEY, H. E. (1927). The effect of splenectomy on the course of *Spirochaeta recurrentis* infection in squirrels. *Abh. a. d. Gebiet d. Auslandskunde, Hamburg. Univ.*, **26**, 328.

MESNIL, F. (1908). [Review of the papers by Brumpt and Doreau cited above.] *Bull. Inst. Pasteur*, **6**, 964.

NÄGELSBACH, E. (1934). Aerztliche Erfahrungen im Hochland von Abessienien. *Arch. f. Schiffs- u. Trop.-Hyg.*, **38**, 100.

NICOLLE, C. (1932). Deux problèmes nouveaux de diagnostic différentiel, celui des fièvres exanthématiques et celui des fièvres récurrentes. *Bull. Inst. Pasteur*, **30**, 961.

PAOLI, P. DE (1930). Osservazioni sopra un focolaio epidemico di febbre ricorrente in Eritrea. *Arch. Ital. Sci. Med. Colon.*, **11**, 303.

RIDING, D., and MACDOWELL, T. W. (1927). Preliminary notes on relapsing fever in the Anglo-Egyptian Sudan. *Trans. Roy. Soc. Trop. Med. & Hyg.*, **20**, 524.

RUBINSTEIN, P. L. (1930). Ueber die Natur der Immunität beim Rückfallfieber, XII. *Ztschr. f. Immunitätsforsch.*, **65**, 538.

——— and GOLUBEWA, E. E. (1930). Ueber eine noch unbekannte Funktion des retikuloendothelialen Systems. *Ibid.*, **66**, 1.

RUSSELL, H. (1931). Human and experimental relapsing fever, Accra, Gold Coast, 1929-1930. *W. Afr. Med. Jl.*, **4**, 59.

SANARELLI, G. (1927). Identité entre spirochètes et bacilles fusiformes : les héliconèmes 'vincenti.' *Ann. Inst. Pasteur*, **41**, 679. (Quoted by Hindle, E. (1931) : The spirochaetes ; in 'A System of Bacteriology in Relation to Medicine,' vol. 8, chapter IV, p. 101. London.)

SERGENT, EDM., and FOLEY, H. (1910). Recherches sur la fièvre récurrente et son mode de transmission dans une épidémie algérienne. *Ann. Inst. Pasteur*, **24**, 337.

SIBILIA, D. (1937). Primo contributo alla conoscenza della *Spirochaeta recurrentis* osservata in Addis Abeba. *Policlinico* (*sez. prat.*), **44**, 772.

TOMIOKA, Y. (1924). Experimenteller Beitrag zur Frage der Immunität bei Recurrens und ihrer Beeinflussung durch die Salvarsantherapie. *Zentralbl. f. Bakt., Orig.*, **92**, 41.

VELU, H., BALOZET, L., and ZOTTNER, G. (1931). Influence de la splénectomie dans l'infection expérimentale par *Spirochaeta hispanicum*. *C. R. Soc. Biol.*, **106**, 890.

WENYON, C. M. (1926). Protozoology. vol. 2. London.

YUAN-PO, LI (1933). A new method for the cultivation of *Spirochaeta recurrentis*. *Kitasato Arch. Exp. Med.*, **10**, 78.

——— (1936). Méthode de préparation simplifiée de mon milieu pour la culture du *Spirochaeta recurrentis*. *Ann. Parasitol.*, **14**, 76.

PLATE IX

EXPLANATION OF PLATE IX

Fig. 1. Polymorphous appearance of the spirochaetes in stained blood-films. (×1,900.)

Fig. 2. Appearance of the spirochaetes in films of frozen blood. (×1,900.)

Fig. 3. Appearance of the spirochaetes in the haemocoele fluid of the louse. (×1,900.)

Fig. 4. Large tangled mass of spirochaetes in the haemocoele of the louse. (×1,900.)

PLATE IX

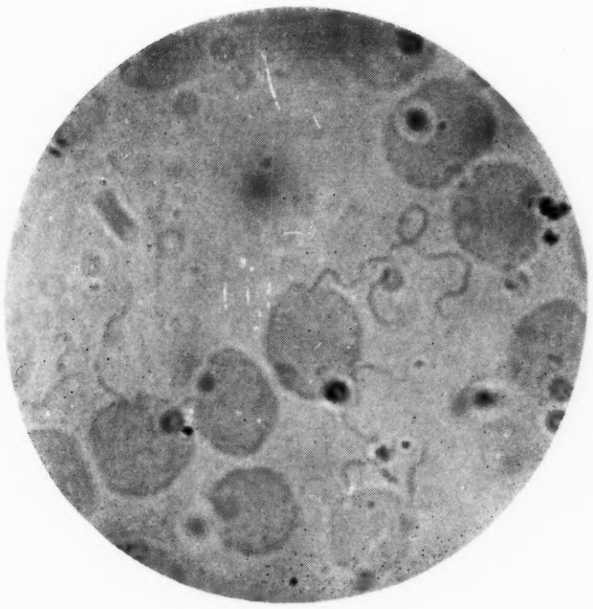


Fig. 1

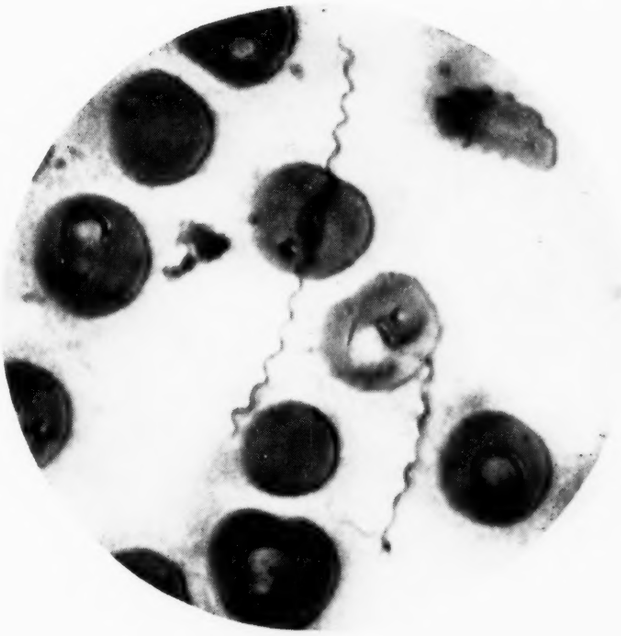


Fig. 2

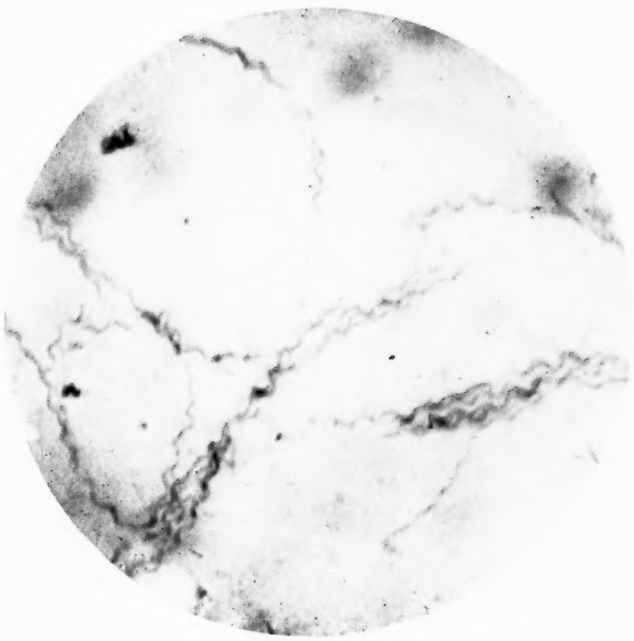


Fig. 3

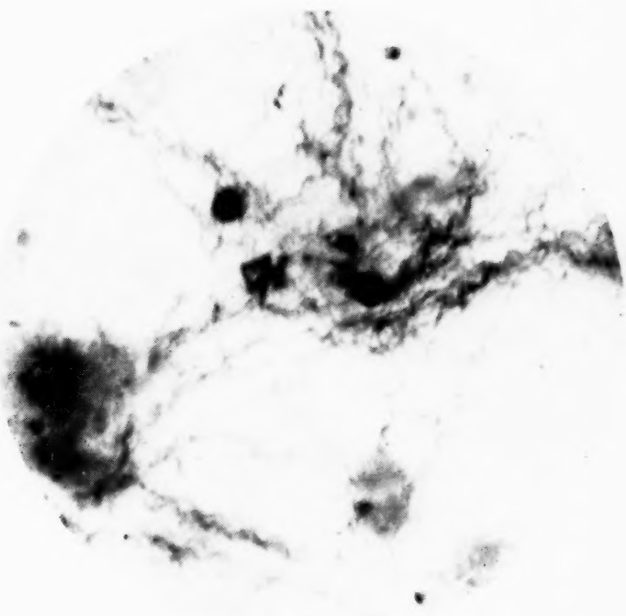


Fig. 4

H. R. Grubb, Ltd., Poplar Walk, Croydon

UNIV. OF MICH.

THE NON-TRANSMISSION OF ABYSSINIAN LOUSE-BORNE RELAPSING FEVER BY THE TICK *ORNITHODORUS SAVIGNYI* AND CERTAIN OTHER BLOOD-SUCKING ARTHROPODS

BY

R. KIRK*

(*From the Stack Medical Research Laboratories, Khartoum, Anglo-Egyptian Sudan*)

(*Received for publication September 17th*, 1938)

INTRODUCTION

Many years ago it was suggested by Nicolle and Anderson (1926) that the epidemiology of relapsing fever would ultimately be clarified by the discovery of some reservoir—most probably a tick—in which the louse-borne virus is able to persist for long periods independently of the human host. This hypothesis would obviously be strengthened if it could be shown experimentally that the louse-borne strains were able to persist in ticks and to be transmitted once more to susceptible animals through the medium of these arthropods. But the carefully devised experiments of Nicolle, Blaizot and Conseil (1913) to transmit the Tunisian strain by means of *Ornithodorus savignyi* gave only negative results, and other workers have recorded similar failures. Sergent and Foley (1910) were unable to transmit the Algerian strain by means of *Argas persicus*. Brumpt (1908) failed to transmit either this strain or the American strain by *Ornithodorus moubata*. Balfour (1911*a*) recorded negative attempts with the Egyptian strain and *Ornithodorus savignyi*, and Ingram (1924) was unable to transmit the Gold Coast strain by *Ornithodorus moubata*. More recently, Brumpt (1936*b*) and Feng and Chung (1937) failed independently to transmit the Chinese strain by *Ornithodorus moubata*.

On the other hand, it is recorded that Manteufel (1908), Neumann (1909), and Schuberg and Manteufel (1910) succeeded in transmitting the Russian and American strains by means of *Ornithodorus moubata*. I have been unable to consult the original papers of these authors, but Brumpt (1936*b*), criticizing their work, points out that nothing is known of the conditions under which these experiments were carried out. The incubation periods in the rats infected by tick bites were apparently unduly long, and, as the spirochaetes were shown to be readily transmissible by rat-lice, Brumpt suggests that the infection was transmitted in reality by these parasites, and not by the bites of the *Ornithodorus*

*The writer is indebted to the Director of the Sudan Medical Service for permission to publish this article.

ticks. A further criticism may be made of the experiments of Neumann and Manteufel in that there seems to be some doubt as to the origin of the strains studied by these authors. The ' Russian strain ' studied by them was in all probability the so-called Frankfurt strain which has been maintained in Germany in rats since 1907, and the original history of which has been lost. More recently Brumpt (1936*c*) concluded from an experimental study of this strain that it was not a true *S. recurrentis*, but a strain of *S. duttoni* which had become modified in various interesting ways by the continuous passage through rats. Nicolle and Anderson (1929) have drawn attention to the confusion which may result from the employment in the laboratory of strains of which the origin is in any way doubtful, and they recommend that such strains should be discarded completely.

In spite of these failures to transmit the louse-borne strains by the intermediary of ticks, the possibility still exists that in some country strains may be found having the special aptitude of surviving in ticks, just as there are some strains which are readily inoculable from man to other animals and some which are not. During the course of the past two years, the writer has had the opportunity of studying a strain of louse-borne relapsing fever of Abyssinian origin. The transmission and other features of the strain have been reported in a separate communication. As louse-borne relapsing fever has only been recognized in Abyssinia within the last few years, and as the organism has not previously been studied in the laboratory, it seemed of interest to ascertain whether or not it was able to persist in ticks, and experiments were therefore carried out to determine, in the first place, whether this strain could persist in *Ornithodorus savignyi*.

REASONS FOR SELECTING *ORNITHODORUS SAVIGNYI*

Ornithodorus savignyi was selected for these experiments for various reasons. In the first place it was readily available in sufficient numbers for experimental purposes. Nicolle, Anderson and Colas-Belcour (1928) have shown that under experimental conditions *Ornithodorus savignyi* can transmit all the varieties of relapsing fever transmissible by *Ornithodorus moubata* ; so that, if this Abyssinian strain could survive in ticks, there was no *a priori* reason to believe that experiments with *Ornithodorus savignyi* were less likely to be successful than those carried out with other ticks.

Ornithodorus savignyi has been cited as an important vector of relapsing fever in Abyssinia (Bruns, 1937 ; Wenyon, 1926), as well as in the neighbouring territory of British Somaliland (Clark, 1937 ; Drake-Brockman, 1915). The evidence for this is rather questionable, but Brumpt (1908) was able to transmit in the laboratory by means of *Ornithodorus savignyi* a tick-borne spirochaete obtained originally from specimens of *Ornithodorus moubata* sent from Abyssinia.

Certain epidemiological features of the disease, as we observed it in the Sudan, tended to throw suspicion on *Ornithodorus savignyi*, which is not a house-

frequenting tick but occurs typically in outdoor situations, in the vicinity of markets, under trees where livestock collect for shade in the heat of the day, and in similar places. The relapsing fever in question occurred almost exclusively among wandering immigrants, who had no house-property or relatives with satisfactory living-accommodation in the districts they passed through. These people commonly took their mid-day rest, and made their camping-grounds for the night, in just such places as are frequented by *Ornithodorus savignyi*.

METHODS

The methods employed consisted essentially in allowing ticks to bite infected persons or animals ; thereafter, at varying periods, attempts were made to infect susceptible animals by the bites of these ticks, by the inoculation of the coxal fluid, and finally by the inoculation of the total contents of the ticks, emulsified in saline. To avoid the many pitfalls which beset experiments of this nature, particular attention was given to details, the importance of which is emphasized by consideration of previous experiments recorded in the literature.

The common grey monkey of the Sudan (*Cercopithecus sebaeus*) was chosen as the animal most likely to be infected from the ticks, because, of the animals at our disposal, it had already been found to be the most susceptible to the disease. It was important that the animals used should be as sensitive as possible to the infection, in order to obviate light infections which might be missed, or inapparent infections of the type found by Velu, Balozet and Zottner (1930) in rabbits. With this in view, the animals were splenectomized, as our own observations and those of other workers had shown that the effect of this operation was, if anything, to increase the susceptibility to relapsing fever. The monkeys were obtained with the help of local natives from the woods of the Sennar and Singa districts of the Sudan, and were all young animals. Nicolle and Anderson (1927*b*) say that no wild animals should be used for experiments with relapsing fever, as many of them may harbour natural spirochaetal infections. It was fully realized that these monkeys were the same species as that in which Plimmer (1912) found spirochaetes in the London Zoo, and that Ranken (1912) has recorded from the Southern Sudan a natural spirochaetal infection in the allied *Cercopithecus ruber*. Except for white mice, however, no laboratory-bred animals had been found susceptible. A few experiments were carried out with white mice, but it is doubtful whether even these can be regarded as absolutely safe, since Vinzent (1927) has recorded the spontaneous appearance of a spirochaete in a ' clean ' laboratory-bred strain of mice after inoculation with trypanosomes. The blood of all our monkeys was examined before they were used for experiment, to exclude any which showed spirochaetes, but no positives were found. During some earlier work with this spirochaete a monkey was found which appeared to be quite insusceptible to the strain. Because of this, the further precaution was taken of proving that the animals used in the

transmission experiments were susceptible, before negative results were accepted. This was done by inoculating them with blood containing spirochaetes after the conclusion of the transmission experiments.

All the monkeys were found to be naturally infested with lice (*Pedicinus* sp.). Although attempts to transmit the spirochaetes experimentally by these lice were unsuccessful, precautions were taken to segregate monkeys which had been infected, so as to exclude the possibility of any transference of ectoparasites from infected to healthy animals. It may be recalled that Brumpt (1936*b*) has discredited the experiments of Neumann and Manteufel on the grounds that this precaution was probably omitted. The animal-houses in Khartoum are mosquito-proof, thus obviating the possibility of mechanical transmission by *Stomoxys* (Schuberg and Kuhn, 1911), and tabanids, which are common enough in the vicinity.

As Hindle (1911) and Kleine and Krause (1932) have shown that a certain proportion of *Ornithodorus* seem to be insusceptible to spirochaetal infection, care was taken to use a sufficient number of ticks to exclude fallacy arising from this cause.

Nicolle, Anderson and Colas-Belcour (1928) maintain that a vicarious vector can often be infected in the nymphal stages, although the adults are refractory, and they stress the necessity of using nymphs as well as adults in transmission experiments. The necessity for this is denied by Kleine and Krause (1932), and also by Kritschewski and Dvolaitskaya-Barischewa (1931); but, in order to ensure that the optimum possible conditions for the infection of the ticks were presented, a certain proportion of nymphs were included in the transmission experiments.

As the experiments were designed to find out whether *Ornithodorus savignyi* could act as a true vector, and not whether mere mechanical transmission was possible, it was necessary to ensure that sufficient time elapsed between the infective feed and any attempt to transmit the spirochaete to a new host by means of the tick. Hindle (1911) has shown that the spirochaetes may persist up to four weeks in the alimentary canal of the tick after a feed. At least one month was allowed to elapse, therefore, except in the case of the first two batches of ticks, when feeding experiments only were carried out after the lapse of a lesser period. Tissue emulsions from the ticks were not inoculated in any instance until at least a month had elapsed from the date of the infective feed.

Borrel and Marchoux (1905), and later Marchoux and Couvy (1913), have stated that *Argas* loses its power to transmit fowl spirochaetes if kept in the cold, but regains it after 2–3 days at 30°–35° C. We therefore made a practice of keeping the ticks in the incubator (37° C.) for at least five days immediately before trying to infect animals from them. Although Brumpt (1936*a*), as a result of experiments and observations in nature, disagrees with the findings of Borrel and Marchoux, Hindle (1911) has shown that this procedure of 'warming' the ticks for a few days results in an active development and

multiplication of the spirochaetes, thereby making them more readily visible in the secretions of the tick, or in its tissues during dissections.

In the earlier attempts of the writer to transmit the spirochaete by ticks, feeding experiments, examination of the coxal fluid, dissections and examinations of the tissues of the ticks were carried out. This was a very tedious process, and it was often difficult to induce the ticks to feed in the required number at a given time. It was found that the only satisfactory method entailed having the host-animal spread-eagled on a board and fairly tightly tied down. In an early experiment, when attempts were being made to induce ticks to bite a mouse, the mouse bit and killed two of the ticks. After a number of negative results these detailed methods were discarded, and reliance was placed on the simple inoculation of the total contents of the tick. This was obtained by opening the tick dorsally, and removing all the soft parts. A saline emulsion of these soft tissues was made, Griffiths' tubes being found particularly suitable for this purpose. The emulsion was filtered through glass wool and inoculated into the animal. The subcutaneous route was always used, as the material inoculated was certain to be contaminated with organisms of various sorts. No abscesses resulted, however, although by the time the experiments were concluded two of the monkeys had indurated areas in the subcutaneous tissues at sites where several inoculations had been given.

Nicolle and Anderson (1927*a*) have pointed out that infection following the inoculation of the tissues of a supposed vector cannot be regarded as evidence that the latter is capable of transmitting the disease in nature. This objection would have been of prime importance had our results been positive, but as they were consistently negative this method provided the most convincing proof that the spirochaetes had completely disappeared from the tissues of the ticks. A supply of ticks was held in reserve to be used as controls in the event of a positive transmission being recorded, but none was required. Batches of ticks from Khartoum, El Obeid and Kassala were dissected and smears were made from their tissues ; but no sign of spirochaetes was at any time encountered.

The following is a brief résumé of the transmission experiments carried out with *Ornithodorus savignyi*.

(*a*) *Feeding Experiments*

Batch A. November 13th, 1937. Twelve ticks fed on a case of relapsing fever in Sennar ; 2 ticks found dead three days after the feed.

Batch B. November 13th, 1937. Twelve ticks fed on a second case in Sennar ; 1 tick died three days after the feed.

Batch C. November 22nd, 1937. Twelve ticks fed in a case in Kassala ; 1 tick died.

Batch D. November 22nd, 1937. Twelve ticks fed on a second case in Kassala.

Batch E. November 25th, 1937. Twelve ticks fed on a third case in Kassala. This case was a definite relapse ; 1 tick found dead four days after the feed.

Batch F. December 26th, 1937. Eighteen ticks and 30 nymphs fed on a monkey (no. R2) during first attack of fever after inoculation from a case in Sennar ; 2 ticks died three days after the feed.

Batch G. December 27th, 1937. Sixteen ticks and 30 nymphs fed on another monkey (no. R1) inoculated from the same case in Sennar ; 1 tick died.

Batch H. Eight ticks : 4 were fed on November 27th, 1937, on two mice inoculated from monkey no. R1 ; the other 4 were fed on December 28th, 1937, on two mice inoculated from monkey no. R3, which was infected from monkey no. R1.

Batch J. Eighteen ticks and 20 nymphs fed on monkey no. R2, inoculated from a case in Sennar. The monkey relapsed, and the ticks of this batch were fed during the relapse.

(*b*) *Attempted Transmissions*

December 3rd, 1937. Eight ticks, 4 from batch A and 4 from batch B, were allowed to bite a monkey (no. R4), and the coxal fluid which was exuded at the time of the bite was sucked up into a syringe and inoculated subcutaneously into the monkey.

December 4th, 1937. Three ticks from batch B were allowed to bite a white mouse, and the coxal fluid was inoculated into the mouse. Two ticks from batch A were lost by being killed by the mouse.

December 10th, 1937. Four ticks from batch A were induced to bite two white mice, and the coxal fluid was inoculated subcutaneously. The ticks were dissected, and smears were made from the gut, Malpighian tubules and the haemocoele fluid. Fungal bodies only discovered ; no spirochaetes.

December 14th, 1937. Five ticks from batches A and B were allowed to bite a monkey (no. R4). Coxal fluid inoculated subcutaneously. Ticks dissected, smears from gut, Malpighian tubes and haemocoele negative, except for fungal organisms. Total contents of ticks inoculated into another monkey (no. R5).

December 20th, 1937. Six ticks from batches A and B allowed to bite monkey no. R4. Coxal fluid inoculated. Smears made from gut, haemocoele and Malpighian tubes of 4 ticks : all negative for spirochaetes. Total contents of 10 ticks inoculated into monkey no. R5.

December 26th, 1937. Total contents of 11 ticks (batch C) inoculated into two monkeys (nos. R5 and R6).

January 12th, 1938. Total contents of 12 ticks (batch D) inoculated into monkeys no. R5 and R6.

January 25th, 1938. Total contents of 19 ticks (batches E and H) inoculated into monkeys no. R5 and R6.

January 30th, 1938. Total contents of 16 ticks (batch F) inoculated into monkeys no. R5 and R6. Smears from gut, haemocoele and Malpighian tubes of 2 ticks from this batch were examined and found negative for spirochaetes.

February 2nd, 1938. All the nymphs of batches F and G ground up in saline, and inoculated into monkeys no. R5 and R6.

February 8th, 1938. Nymphs and contents of ticks (16) of batch J emulsified in saline and inoculated into monkeys no. R5 and R6.

RESULTS

The results of these experiments were entirely negative, although a variety of strains were used, including relapse strains as well as those from the original infection, and strains which had been passed through monkeys and mice, as well as strains taken directly from human patients. The blood of the animals whose infection was attempted was examined daily during the course of the experiments, and for 20 days after the last attempt was made to infect them, but spirochaetes were never found, although the animals were later shown to be susceptible by the inoculation of infected blood. Nor were spirochaetes found

either by dark-ground examination of the coxal fluid exuded by the ticks, or in stained smears from the tissues of the ticks.

A curious organism was almost invariably found in the tissues of the ticks, more particularly in the Malpighian tubes. Sometimes this organism appeared as bacillary forms of varying length ; at other times, it had a filiform septate appearance, and resembled the mycelium of a fungus more than anything else. The organism was found in ticks which had not fed on infected blood, as well as in those used in the experiments. On two occasions attempts were made to remove a piece of Malpighian tube under aseptic conditions, and to grow the organism from it in broth. In both instances a fungus appeared in pure culture in the broth. In its general features this fungus so closely resembled some of the ordinary air-borne fungi which are found everywhere in the tropics that it was difficult, without the opinion of a competent mycologist, to be sure that it was not a contamination. Many years ago Balfour (1911*b*) observed what appears to have been the same organism in these ticks, but the present writer has found no other reference to it. Possibly it is a symbiotic organism, such as have been found in a great number of other haematophagous insects.

Another interesting fact noticed during these experiments was that a certain number of ticks died some 2–3 days after a meal of infected blood. Smears always showed spirochaetes in the gut of these ticks after death, similar in appearance to those found in the blood, and where dark-ground examination was carried out the spirochaetes were motile. No attempt was made to investigate the reason for these deaths among the ticks. The observation is merely recorded, and it is not possible at present to say whether the deaths were due to the spirochaetes, to the blood from an unusual host, or to some other reason.

EXPERIMENTS WITH OTHER ARTHROPODS

Argas persicus. One attempted transmission was carried out with *Argas persicus*, a tick which has at various times been cited as the vector of human relapsing fever in different parts of the world, all on very questionable evidence (Manson-Bahr, 1929). On January 8th, 1938, eight adult *Argas* were induced to feed on a monkey which had numerous spirochaetes in the peripheral blood. Six weeks later the soft tissues of these ticks were emulsified in saline, and inoculated into two white mice, but no infection resulted.

Pedicinus. Some observations were made with regard to the ability of the lice found naturally on the monkeys to transmit the disease. During the experiments with *Ornithodorus*, as stated above, care was taken to prevent possible infection of one monkey from another by the agency of these lice, as Manteufel and Neumann had found the European strain easily transmissible by rat-lice. Similarly, Chung (1937) found that the Chinese strain was readily transmissible from one squirrel to another by means of the lice occurring naturally on these animals. On January 4th, 1938, lice were collected

from a monkey which had spirochaetes in its blood on that day, and, in addition, had had a 3-day attack some 10 days earlier. Twelve lice were collected, of which six were examined microscopically, and six, after a fast of 24 hours, were ground up in saline, and inoculated into a gerbille. The gerbille did not develop an infection. In the smears of the lice spirochaetes were found in fair numbers, but in their shape and disposition they resembled those seen in the blood rather than the metacyclic forms, although they were relatively more numerous than the red blood corpuscles, which had presumably been digested.

Sixteen days later a further six lice were collected from this monkey, and another attempt was made to infect the gerbille, but it was unsuccessful.

On March 20th, 1938, eight lice were collected from another monkey which had had spirochaetes in its blood some 20 days earlier. These lice were ground up in saline and inoculated percutaneously into a splenectomized monkey, but no infection resulted. On this occasion the lice were not examined microscopically.

Ten lice were collected from another monkey (no. R4), which died of experimental relapsing fever after having had spirochaetes in the blood for eight consecutive days. Abundant spirochaetes were found in these lice, even when no blood corpuscles were observed. These spirochaetes, however, resembled the forms seen in human blood. They were much thicker and more irregular than the metacyclic forms seen in *Pediculus*, and, as far as one could make out, they were restricted to the alimentary canal. In some of the preparations, the appearances suggested that the spirochaetes were actually boring their way into the cells of the gut, coiling up inside them, and breaking up into granules. It is impossible to say whether this represents invasion of the tissues by living organisms, or merely phagocytosis of the spirochaetes. The negative transmission experiments rather suggest the latter; but they are comparatively few in number, and it appears to be rather more difficult to obtain positive transmission with lice than with ticks. Wenyon (1926) remarks on the scarcity of experimental transmissions from the louse. In the cells of *Pedicinus*, bacteria were also constantly present. They were, however, easily distinguishable as such, and not likely to be confused with disintegrating spirochaetes.

REFERENCES

BALFOUR, A. (1911*a*). The spirochaete of Egyptian relapsing fever: is it a specific entity? *4th Rep. Wellcome Trop. Res. Lab.*, A, 71.

——— (1911*b*). Spirochaetosis of Sudanese fowls. *Ibid.*, A, 80.

BORREL, A., and MARCHOUX, E. (1905). *Argas* et spirilles. *C. R. Soc. Biol.*, **63**, 362.

BRUMPT, E. (1908). Transmission du *Spirochaeta duttoni* par l'*Ornithodorus savignyi*; transmission du *Spirochaeta duttoni* et du *Spirochaeta gallinarum* par l'*Ornithodorus moubata*, non transmission des spirochètes de la fièvre récurrente américaine et algérienne par ce même parasite. *Bull. Soc. Path. Exot.*, **1**, 577.

——— (1936*a*). Action nulle du froid sur le pouvoir infectieux des argasinés vecteurs de fièvres récurrentes. *Ann. Parasitol.*, **14**, 436.

——— (1936*b*). Le spirochète (*Spirochaeta recurrentis*) de la fièvre récurrente à poux de Chine, n'est pas transmis expérimentalement par la piqûre de l'*Ornithodorus moubata* et ne se conserve pas chez cet acarien. *Ibid.*, **14**, 578.

BRUMPT, E. (1936*c*). Sur l'identification des spirochètes récurrents : étude d'une souche de *Spirochaeta recurrentis* (?) conservée depuis longtemps sur souris, dans divers laboratoires d'Allemagne. *Ibid.*, **14**, 589.

BRUNS, A. (1937). Ueber Rückfallfieber in Abessinien. *Arch. f. Schiffs- u. Trop.-Hyg.*, **41**, 343.

CHUNG, H. L. (1936). Studies on the transmission of relapsing fever in north China. I : Preliminary observations. *Chinese Med. Jl.*, **50**, 1723.

CLARK, E. M. (1937). Report on relapsing fever in Burao. *Somaliland Protectorate Ann. Med. & San. Rep. for* 1936, App. II, 57.

DRAKE-BROCKMAN, R. E. (1915). On an epidemic of African tick fever among the troops of British Somaliland. *Trans. Soc. Trop. Med. & Hyg.*, **8**, 201.

FENG, L. C., and CHUNG, H. L. (1937). Attempts to infect *Ornithodorus moubata* with the Chinese strain of *Spirochaeta recurrentis*. *Proc. Soc. Exp. Biol. & Med.*, **36**, 330.

HINDLE, E. (1911). The transmission of *Spirochaeta duttoni*. *Parasitol.*, **4**, 133.

INGRAM, A. (1924). *Ornithodorus moubata*, Murray, in relation to relapsing fever in the Gold Coast. *Ann. Trop. Med. & Parasitol.*, **18**, 95.

KLEINE, F. K., and KRAUSE, M. (1932). Zur Uebertragung der Rückfallfieberspirochäten durch Zecken. *Arch. f. Schiffs- u. Trop.-Hyg.*, **36**, 587.

KRITSCHEWSKI, I. L., and DVOLAITSKAYA-BARISCHEWA, K. M. (1931). *Ornithodorus papillipes* als Ueberträger von Spirochäten des Rückfallfiebers unter experimentellen Bedingungen. *Zentralbl. f. Bakt., Orig.*, **121**, 421.

MANSON-BAHR, P. H., ED. (1929). Manson's Tropical Diseases. 9th ed. p. 168. London.

MANTEUFEL, P. (1908). Weitere Untersuchungen über Rückfallfieber. *Arb. a. d. K. Gesundheitsamte*, **29**, 337. (Quoted by WENYON, 1926.)

MARCHOUX, E., and COUVY, L. (1913). Argas et spirochètes (premier mémoire) : les granules de Leishman. *Ann. Inst. Pasteur*, **27**, 450.

NEUMANN, R. O. (1909). Ueber das Verhalten der Spirochäten des Rückfallfiebers in Tierkörper und die experimentelle Ueberträgung der Parasiten durch Zecken und Läuse. *Münch. Med. Woch.*, **56**, 477. (Quoted by WENYON, 1926.)

NICOLLE, C., and ANDERSON, C. (1926). Fièvre récurrente transmise à la fois par ornithodores et par poux. *C. R. Acad. Sci.*, **182**, 1450.

——— ——— (1927*a*). D'une erreur commune dans la conduite des expériences portant sur la détermination des agents de transmission des spirochétoses et de la même erreur dans toutes les investigations analogues. *Arch. Inst. Pasteur de Tunis*, **16**, 228.

——— ——— (1927*b*). De certaines règles à suivre dans l'expérimentation sur les spirochétoses sanguines. *Ibid.*, **16**, 333.

——— ——— (1929). Sur la nécessité de l'identification et d'un contrôle des spirochètes récurrents entretenus dans les laboratoires. *Ibid.*, **18**, 73.

——— ——— and COLAS-BELCOUR, J. (1928). Adaptation expérimentale de spirochètes récurrents à des ornithodores autres que ceux qui les transmettent dans la nature. *C. R. Acad. Sci.*, **187**, 1105.

——— BLAIZOT, L., and CONSEIL, E. (1913). Étiologie de la fièvre récurrente : son mode de transmission par les poux. *Ann. Inst. Pasteur*, **27**, 204.

PLIMMER, H. G. (1912). On certain blood parasites. *Jl. R. Micr. Soc.*, 133.

RANKEN, H. S. (1912). Note on the occurrence of a spirochaete in *Cercopithecus ruber*. *Brit. Med. Jl.*, **1**, 1482.

SCHUBERG, A., and KUHN, P. (1911). Ueber die Uebertragung von Krankheiten durch einheimische stechende Insekten, I. *Arb. a. d. K. Gesundheitsamte*, **31**, 377. (Quoted by Hindle, E. (1931) : Blood spirochaetes ; in ' A System of Bacteriology in Relation to Medicine,' vol. 8, chapter VI, p. 147. London.)

——— and MANTEUFEL, P. (1910). Ueber erworbene Immunität gegen Rekurrens bei *Ornithodorus moubata*. *Ztschr. f. Immunitätsforsch.*, **4**, 512. (Quoted by Brumpt, 1936*b*).

SERGENT, EDM., and FOLEY, H. (1910). Recherches sur la fièvre récurrente et son mode de transmission dans une épidémie algérienne. *Ann. Inst. Pasteur*, **24**, 337.

VELU, H., BALOZET, L., and ZOTTNER, G. (1930). Neurotropisme de *Spirochaeta hispanicum* (souche de Mansouriah) pour le lapin : forme nouvelle de l'infection inapparente. *C. R. Soc. Biol.*, **103**, 381.

VINZENT, R. (1927). Spirochétose de la souris blanche et infection mixte trypano-spirochétique. *Ann. Inst. Pasteur*, **41**, 131.

WENYON, C. M. (1926). Protozoology. vol. 2, p. 1249. London.

A QUANTITATIVE STUDY OF THE PHOTOSENSITIVITY INDUCED IN TRYPANOSOMES BY ACRIFLAVINE

BY

FRANK HAWKING*

(*From the Department of Pharmacology, Welsh National School of Medicine, Cardiff*)

(*Received for publication July 26th*, 1938)

When trypanosomes have absorbed acriflavine, they become photosensitive, as was first described by Jancsó (1931), who brought them into contact with the drug *in vivo* and exposed them to strong light on the stage of a dark-ground-illumination microscope, under which circumstances they soon became motionless and presumably dead. The purpose of the present paper is to describe a quantitative investigation of this technique, so that it can be used to measure the quantity of acriflavine in the trypanosomes or in the medium with which they are in contact.

As described in a recent paper (Hawking, 1938), the pharmaceutical preparations of 'acriflavine' (which were used for the present work) really consist of about 60–75 per cent. diamino-methyl-acridinium-chloride hydrochloride and about 25–40 per cent. diamino-acridinium hydrochloride, the former being about four times as active in producing photosensitivity as the latter. According to Hausmann and Krumpel (1929) the absorption of light by 'trypaflavin' is maximal at wavelengths 458$\mu\mu$ and again at 165$\mu\mu$, and minimal at 321·5 and 235$\mu\mu$; the wavelengths absorbed are presumably those which excite the fluorescence. When exposed to ultra-violet illumination, diamino-methyl-acridinium-chloride emits light of wavelengths 460–640$\mu\mu$, maximum at 490$\mu\mu$; and diamino-acridine emits light of the same wavelengths but only half the intensity (Oesterlin, 1936). The visible spectrum extends from 380 to 800$\mu\mu$.

TECHNIQUE

The miscroscope used was a Leitz-Wetzlar fitted with a dark-ground-illumination condenser. Observations were made with the 1/6 objective, which gives magnification ample for this purpose and which is more convenient than the oil-immersion lens. The source of light was a Pointolite lanp with glass condenser, the distances of the lamp and of the condenser from the microscope both being standardized. Determinations are made by focussing the condenser to give a small area of brilliant illumination in the centre of the field. Suitable individual trypanosomes are identified in the poorly illuminated peripheral portion of the field and are moved into the bright area. The period of illumination required before the trypanosome becomes motionless is determined by a stop-watch.

*Gillson Scholar of the Society of Apothecaries of London.

The exact focussing of the condenser is of the greatest importance, and the difficulty of obtaining exactly equal intensities of illumination with different slides is probably the principal source of error in these determinations. The condenser was always raised or lowered until the position of maximum concentration of light was obtained for each slide; but, since active ultra-violet rays may come to focus on a slightly different plane from those of the visible light, it is difficult to be certain that the standard (maximum) illumination has always been exactly obtained. Errors due to this source may be somewhat minimized by measuring the photosensitivity of a number of trypanosomes, then refocussing the condenser and making further measurements, repeating the focussing a third time if desired; by this means an average of several positions of the condenser is obtained. Perhaps a more satisfactory standardization of the illumination might be obtained by bringing the condenser to a standard position, as determined by a scale and vernier, and by using slides of an accurately standardized thickness.

The thickness of the film should be as small as possible, without the coverslip actually pressing on the trypanosomes and distorting them; but moderate variations of thickness do not seem greatly to influence the result.

The slides also should be of uniform thickness and colour, although in actual practice differences in this respect appear to be of minor importance. In the present work, ' half-white ' slides were used. The density of the trypanosome-suspension should be standardized; if the concentration is much greater than 2,000 per mm.^{3} the photosensitivity will be inversely proportional to the number of organisms present (see below).

The duration of contact between drug and trypanosome seems to be immaterial, provided that sufficient time has elapsed for equilibrium between the organism and the medium to have been reached (for which, 20 minutes should allow ample excess), and provided that the time is not so long that the trypanosomes are beginning to be killed; most of the concentrations here studied lay well below the level required to produce death in a few hours. The determinations of the present paper were made after half to one-and-a-half hours' contact. More prolonged contact, up to 24 hours, did not produce any constant change in the photosensitivity.

The temperature of contact is also better standardized, but seems relatively unimportant. In a number of parallel determinations at 37° C. and 17° C. respectively, the photosensitivity tended to be slightly greater if the mixture had been kept at room-temperature than if it had been incubated at 37° C., but the difference was not constant and was hardly large enough to be considered significant. In any case, the actual temperature at which the determinations are made is that of the microscope stage, which is presumably at room-temperature.

While awaiting examination, the tubes containing acriflavine are naturally kept in the dark or in a shaded place.

In the experiments described below, the figures given are based on averages of usually about 10 individuals, except with the longer durations, when observations become wearisome and time-consuming and when fewer individuals have been counted. If a single individual in a group required a much shorter, or longer, exposure than the rest of the group, it was ignored, as being a rare extreme, the inclusion of which in a small group would only distort the average.

When ordinary trypanosomes are examined under dark-ground-illumination with the oil-immersion objective, the cell-outline and flagellum show moderately brightly ; the cytoplasm contains a number of poorly marked granules, and the parabasal body is visible but indistinct. In the centre of the parasite is a darker area, presumably the nucleus. Trypanosomes which have taken up acriflavine exhibit a much more brilliant appearance ; the cell-outlines, flagellum and undulating membrane shine more brightly ; the cytoplasm contains a number of large luminous granules ; the parabasal body shows up as a luminous granule, similar to those in the cytoplasm, and in some trypanosomes a small dark or pale-bluish vacuole can be seen between the parabasal body and the blepharoplast ; the dark area in the position of the nucleus remains unchanged. These appearances can be seen most easily in the trypanosomes immobilized by the light ; otherwise the movements are too vigorous to permit examination of the details.

When a trypanosome containing acriflavine is exposed to the strong light, its movements are gradually diminished. Then immobilization commences at the posterior end, apparently through coagulation of the protoplasm. No swelling or distortion occurs. The process spreads forwards, but in weak concentrations of acriflavine the end of the flagellum continues to wave spasmodically for several seconds after the rest of the organism is motionless. In such cases the end point is not clearly marked; for measurement, the time at which the trypanosome as a whole appeared dead has been chosen, and a few movements of the tip of the flagellum have been disregarded. Further observation for several minutes after immobilization is complete does not disclose any distortion or disintegration of the parasite, such as occurs with the trypanocidal action of drugs or heat.

Jancsó (1931) named the period in seconds required for the trypanosome to become immobilized, when thus exposed to light, the ' letale Lichtzahl.' In the translation of such terms, it is difficult to steer between an ambiguity and a monstrosity ; this period is here designated as the ' light-index ' (T). The photosensitivity (P), when expressed quantitatively, is the reciprocal of this multiplied by 100.

INDIVIDUAL VARIATION AMONG TRYPANOSOMES

Normal trypanosomes, 3,200 per mm.^{3}, were exposed to acriflavine 0·039 γ per ml. at room-temperature, and the photosensitivity of 100 individuals taken at random was determined. The frequency-distribution of the light-indices is shown in fig. 1, A, the maximum being 21 seconds, the minimum 5, the average

11·6, the standard deviation 3·87, and the coefficient of variation 33·5 per cent. Fig. 1, B, shows the frequency-distribution of resistant trypanosomes, calculated from determinations on 60 individuals of a suspension in contact with acriflavine 0·31γ per ml., the maximum light-index being 35 seconds (apart from a single case of 74 seconds), the minimum 12, the average 21·0, the standard deviation 4·67, and the coefficient of variation 22·2 per cent.

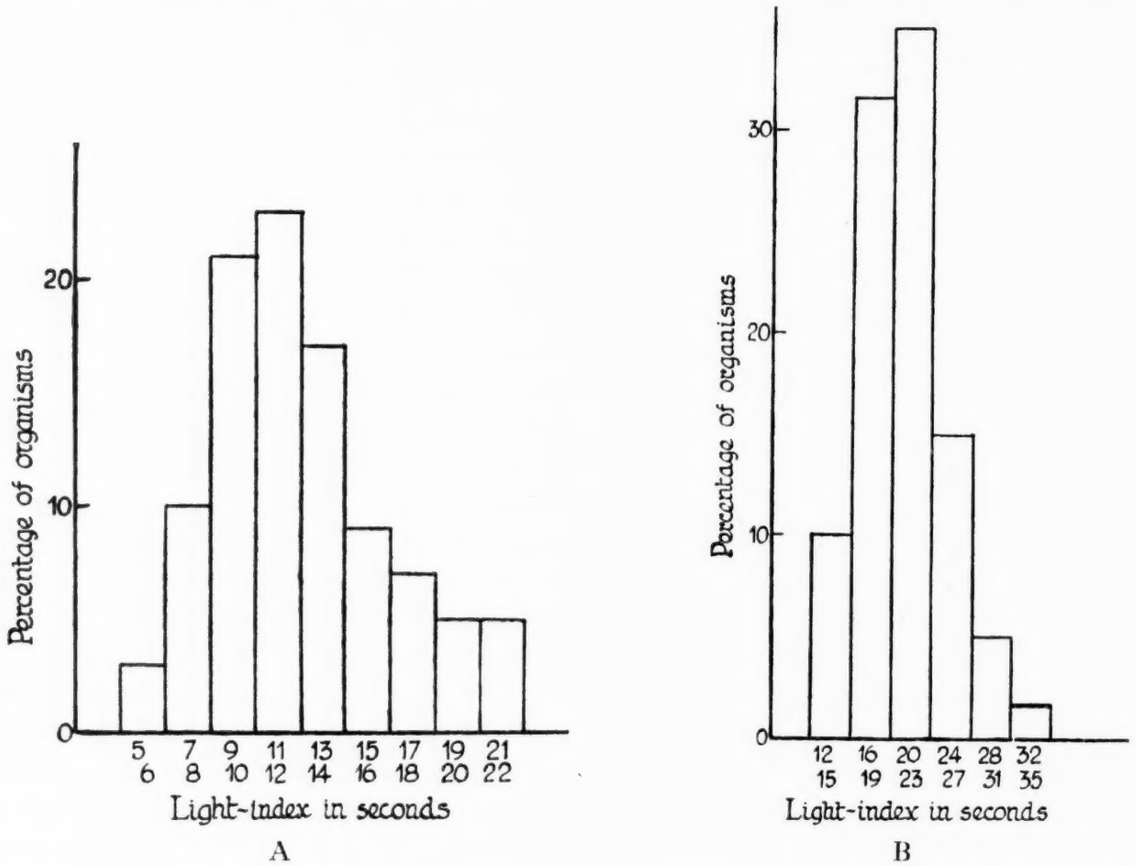


FIG. 1. Frequency-distribution curves for the photosensitivity of trypanosomes exposed to acriflavine.

Abscissae.—Light-index of trypanosomes, in seconds; *Ordinates.*—No. of organisms, percentage of total.

Temperature, 37° C.

A.—Normal strain. Concentration of acriflavine, 0·039γ per ml. Trypanosomes, 3,200 per mm.^{3}

B.—Resistant strain. Concentration of acriflavine, 0·31γ per ml. Trypanosomes, 3,000 per mm.^{3}

Since the average for these resistant trypanosomes is approximately twice that for the normal ones (owing to the relatively weaker concentration of acriflavine), the horizontal scale of fig. 1, B, has been drawn as twice that of fig. 1, A. As is shown in the figures, these frequency-distributions are both slightly askew, but the asymmetry is not great. Classification of the results according to photosensitivity, or logarithm of photosensitivity, produced frequency-distribution curves which were more asymmetrical. It was concluded that the best mean

to take for a group of individual measurements is the average value for the light-index, a procedure which has the additional advantage of being the simplest one to obtain. This has been done in subsequent experiments. The coefficient of variation for the resistant trypanosomes (22·2 per cent.) is smaller than that for the normal trypanosomes (33·5 per cent.), indicating the greater homogeneity of the resistant strain ; this has already been noted in connection with the time-action curves of reduced tryparsamide and of acriflavine described recently (Hawking, 1938).

RELATION BETWEEN PHOTOSENSITIVITY AND NUMBER OF TRYPANOSOMES PRESENT

Experiments were made in which suspensions containing different numbers of trypanosomes were exposed to a standard concentration of acriflavine and the photosensitivity of organisms from the different tubes was determined. Fig. 2

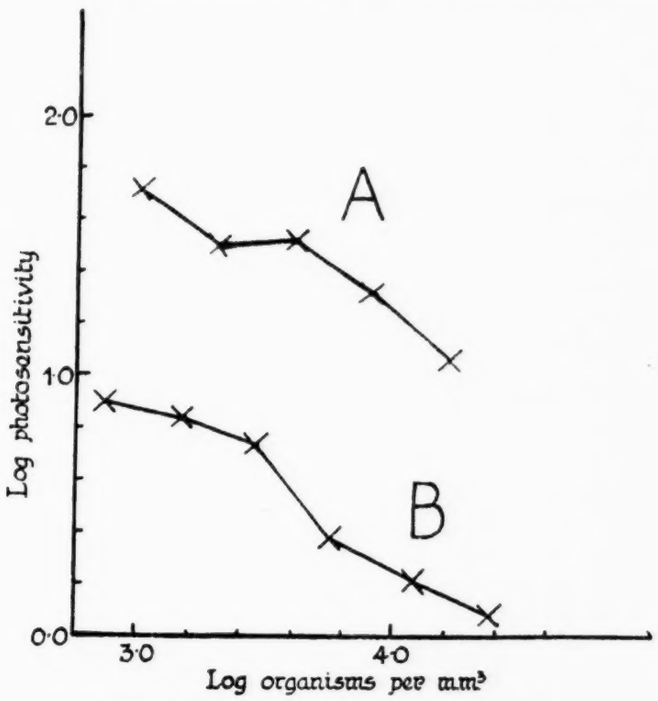


FIG. 2. Showing the relation between the number of normal trypanosomes present and their photosensitivity. A = Acriflavine, 0·078 γ per ml. B = Acriflavine, 0·0195 γ per ml. Temperature, 17° C.

shows the results of two experiments conducted at room-temperature (17° C.) with concentrations of acriflavine 0·078 and 0·019 γ per ml. respectively ; the logarithm of the photosensitivity is plotted against the logarithm of the number of trypanosomes per mm.^{3} The curves show that with suspensions of trypanosomes more numerous than 2,500–4,000 per mm.^{3} (log 3·4-3·6) the photosensitivity diminishes rapidly, proportionately to the increase in the number of organisms present, thus indicating that 2,500–4,000 trypanosomes per mm.^{3}

are sufficient to absorb a large part of the drug present, and that, if still more trypanosomes are added, the amount absorbed per trypanosome is proportionately diminished. Even with suspensions of trypanosomes less than 2,500 per mm.^{3}, there is a slight fall in photosensitivity inversely proportional to the number of organisms, showing that even these numbers of trypanosomes are sufficient materially to reduce its concentration in the medium. The practical conclusion may be drawn that, to obtain comparable results, it is necessary to standardize the number of trypanosomes per mm.^{3}, preferably with less than 2,500 per mm.^{3} In most of these experiments, a suspension of about 1,500 per mm.^{3} has been used.

RELATION BETWEEN PHOTOSENSITIVITY AND INITIAL CONCENTRATION OF ACRIFLAVINE IN MEDIUM

Suitable concentrations of acriflavine were made in nutrient medium, and sufficient trypanosomes were added to bring the final suspension to approximately 1,500 per mm.^{3} The tubes were incubated at 37° C. and the photosensitivity of the organisms was determined in the usual way. The protocol of a typical experiment with normal and resistant trypanosomes respectively is given in Table I, and the results of a series of experiments are shown graphically in fig. 3, in which the logarithm of the photosensitivity is plotted against the logarithm of the initial concentration of acriflavine, γ per ml. This initial

TABLE I

Showing the relation between the initial concentration of acriflavine and the photosensitivity of trypanosomes exposed to it *in vitro*

Tube	Strain of trypanosomes	Concentration of acriflavine : γ per ml.	Average light-index of trypanosomes : seconds	Photosensitivity
1	Normal	0·625	? 1	? 100
2		0·156	4·7	21
3		0·039	19	5·2
4		0·0098	66	1·5
5		0·0025	150	0·67
6		0·00061	270	0·37
7	Resistant	10	? 2	? 50
8		2·5	3·5	29
9		0·625	9·4	11
10		0·156	21·4	4·7
11		0·039	68	1·5
12		0·0098	130	0·77

No. of trypanosomes, 1,500 per mm.^{3} Time of exposure, 30 minutes.
Temperature, 37° C.
Light-index = Period of illumination, in seconds, required to immobilize trypanosomes.
Photosensitivity = 100/light-index.

concentration is not the same as the final concentration with which the trypanosomes come into equilibrium, since, as was shown above, even these light suspensions remove part of the acriflavine, with which they are in contact.

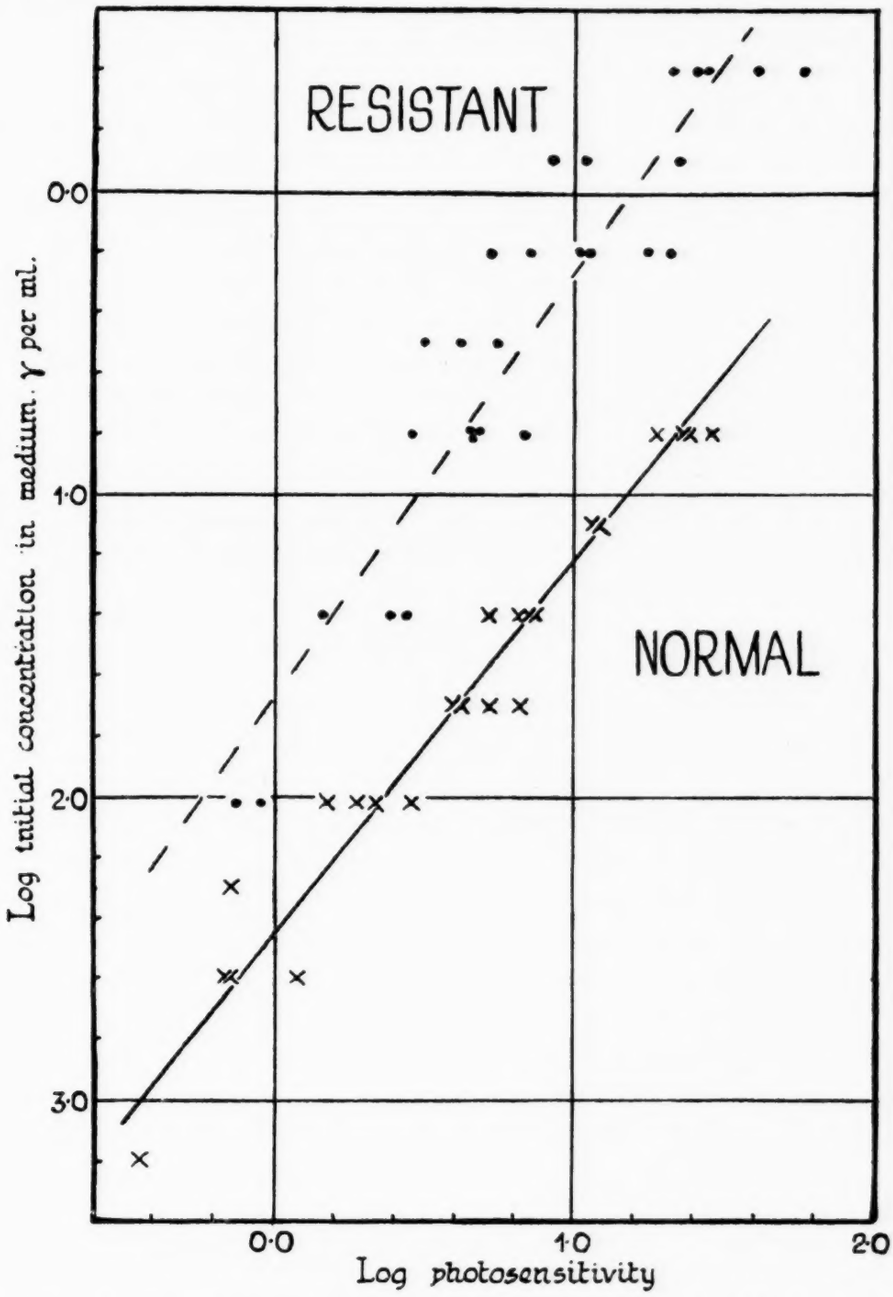


FIG. 3. Showing the relation between the initial concentration of acriflavine in the medium and the photosensitivity of the trypanosomes.
Temperature, 37° C. Trypanosomes, about 1,500 per ml.

From Table I it is seen that for normal trypanosomes the range of concentration within which the photosensitivity can be conveniently measured is from about 0·156 to 0·00061 γ per ml., and for resistant trypanosomes 2·5 to 0·0098 γ per ml.

These concentrations are mostly below the minimum trypanocidal concentration with prolonged exposure, which is about 0·039 γ per ml. for normal trypanosomes and 1·25 γ per ml. for resistant ones.

Fig. 3 shows that, although the points for each strain are a little scattered, they tend to fall along a straight line corresponding to the equation

$$n \log C = \log P + \log K$$

where C is the initial concentration, γ per ml., and P is the photosensitivity, i.e., 100/light-index in seconds. This is another version of the equation $C^n t = K$ used in connection with the time-concentration curves for trypanocidal action (Hawking, 1938). (Presumably $(C-C_o)^n (t-t_o) = K$ would be a more correct form; but C_o is certainly very small—less than 0·0006 γ per ml., the lowest concentration examined—and t_o has also been ignored, since it is probably less than 1 second, and times of less than 2 seconds are difficult to measure by this technique.) As before, n and k are constants, representing the slope and level of the lines respectively. In fig. 3 the value of n has been determined separately for each experiment, and the average of these values has been taken; a line has then been drawn with this slope, so that the sum of the distances, positive and negative, of the individual points from it is zero. Thus, in four experiments with normal trypanosomes, the values for n were 0·80, 0·84, 0·94 and 0·85, average 0·86; and in five experiments with resistant trypanosomes, the values for n were 0·70, 0·68, 0·73, 0·79, 0·71, average 0·72. The corresponding values of log k are $\bar{3}$·97 and $\bar{2}$·81 respectively, and for k, 0·0094 and 0·065. Consequently, for normal trypanosomes the equation is

$$0{\cdot}86 \log C = \log P + \bar{3}{\cdot}97,$$

and for resistant ones

$$0{\cdot}72 \log C = \log P + \bar{2}{\cdot}81.$$

Since the values of n are not identical for the two strains, the lines are not quite parallel; the difference in the values of n appears too large to be due to experimental error, especially as the values in the individual experiments are fairly consistent; but its implications are obscure, all the more so since the significance of n itself is unknown. As with the time-concentration curves, k is the light-index/100 when the concentration is 1 γ per ml., i.e., when log C = O, or the nth power of the concentration when the photosensitivity is unity, i.e., when log P = O. The principal difference between the two lines is in their levels, i.e., the values of log k, corresponding to the different amounts of acriflavine absorbed by normal and resistant trypanosomes respectively.

The chief value of these curves is to afford an empirical relationship between the initial concentration of acriflavine in the medium and the photosensitivity of trypanosomes placed therein (using the present optical system). Then, if the concentration is known, the photosensitivity can be predicted; conversely, the photosensitivity of the trypanosomes can be used to measure the concentration of an unknown solution of acriflavine. The accuracy of such a

technique would depend on the success in standardizing the conditions described above and on the number of determinations ; but with care estimations should be obtained within about 30 per cent. of the correct figure, and the range 0·2–0·001 γ per ml. is much lower than that offered by any other method at present available. The technique could be adapted to small volumes, e.g., 0·1 ml., and no elaborate previous treatment is necessary.

RELATION BETWEEN PHOTOSENSITIVITY AND ACRIFLAVINE-CONTENT OF TRYPANOSOMES

The experiments to determine the relation between the photosensitivity of trypanosomes and their content of acriflavine were performed at room-temperature instead of at 37° C., since the photosensitivity is practically the same at the lower temperature as at the higher, while the trypanocidal action of the compound is delayed.

Experiment

A heavy suspension of normal trypanosomes was mixed with suitable concentrations of acriflavine, and the tubes were placed in the dark. After about 20–40 minutes a small drop of the mixture was taken on a slide, so as to form a thin film, and the photosensitivity of the trypanosomes was determined. For this purpose washing the organisms, to remove acriflavine present in the medium, is better avoided. Even with resistant trypanosomes, the concentration of acriflavine in the medium is many times less than that in the trypanosome, and it can thus be ignored ; on the other hand, if the organisms are washed, part of the acriflavine in the trypanosomes diffuses out and is lost. The trypanosomes were then separated from the fluid by centrifuging, and the concentration of acriflavine in the supernatant fluid was determined by the photosensitivity method. The trypanosomes were extracted with alcohol, and their acriflavine-content measured ; this figure was also obtained, as recently described (Hawking, 1938), by subtracting the amount in the supernatant fluid from that initially present, and it is this value which is used for subsequent calculations. In the case of resistant trypanosomes, a similar procedure was used, but the data are obtained from the amount of acriflavine extracted from the organisms. Table II, A and B, represents a typical experiment with each strain.

Fig. 4 shows the results of a number of experiments arranged as a graph, the logarithm of the photosensitivity being plotted against the logarithm of the acriflavine-content of the trypanosomes. The points are a little scattered, but tend to fall along a straight line according to the equation

$$n \log A = \log P + \log k$$

where A is the acriflavine-content γ per 10^9 trypanosomes, P is the photosensitivity (100/light-index in seconds), and n and k are constants. The two lines given in the figure for normal and resistant trypanosomes respectively have been obtained by taking the average of the values for n in the individual experiments, and by drawing a line of this slope so that the points lie equidistant on either side of it. For normal trypanosomes, n = 1·12 (average of 1·07, 1·10 and 1·19), and log k = 0·211, k being 1·63 ; for resistant trypanosomes, n = 1·12 (average of 0·68, 1·42 and 1·26), and log k = $\bar{1}$·874, k being 0·748. The lines for the normal and the resistant trypanosomes respectively are thus parallel, although,

TABLE II

Showing the relation between the acriflavine-content of trypanosomes and their photosensitivity

A. NORMAL TRYPANOSOMES

Tube	Initial concentration of acriflavine: γ per ml.	Acriflavine in supernatant fluid: γ per ml.	Acriflavine in trypanosomes			Average light-index: seconds	Photo-sensitivity
			Total extracted: γ	Total calculated: γ	Per 10^9 trypano-somes: γ		
1	2·5	0·25	7·6	9·0	31·7	2·5	40
2	1·25	0·11	4	4·6	16·2	5·2	19
3	0·625	—	2	—	7·09	14	7·1
4	0·312	0·038	0·8	1·1	3·90	24	4·1
5	0·156	0·026	0·46	0·52	1·82	59	1·7
6	0·078	0·0054	0·31	0·29	1·03	160	0·64

Volume per tube, 4 ml. Temperature, 16° C. Duration of contact, 25 minutes.
No. of trypanosomes, 70,600 per mm.^{3}
This experiment is shown by triangles in fig. 4.

B. RESISTANT TRYPANOSOMES

Tube	Initial con-centration of acriflavine: γ per ml.	Acriflavine in trypanosomes		Average light-index: seconds	Photo-sensitivity
		Total extracted: γ	Per 10^9 trypanosomes: γ		
1	10	5·1	12·5	3·3	30
2	5	2·95	7·25	5·4	18
3	2·5	1·8	4·42	17	6·0
4	1·25	0·73	1·80	38	2·6
5	0·625	0·5 (?)	1·2 (?)	49	2·0

Volume per tube, 4 ml. Temperature, 16° C. Duration of contact, 45 minutes.
No. of trypanosomes, 102,000 per mm.^{3}
The acriflavine extracted from the trypanosomes was determined by colorimeter in tubes 1–3, and by fluorescence in tubes 4–5.
This experiment is shown by spotted circles in fig. 4.

in view of the wide variation in the values of n for resistant trypanosomes, this is partly coincidence. On the other hand, the line for the resistant strain lies at a lower level than that for the normal strain; part of this difference may be due to the different experimental methods of determining the acriflavine-content of the organisms, mentioned above, but this cannot account for it altogether.

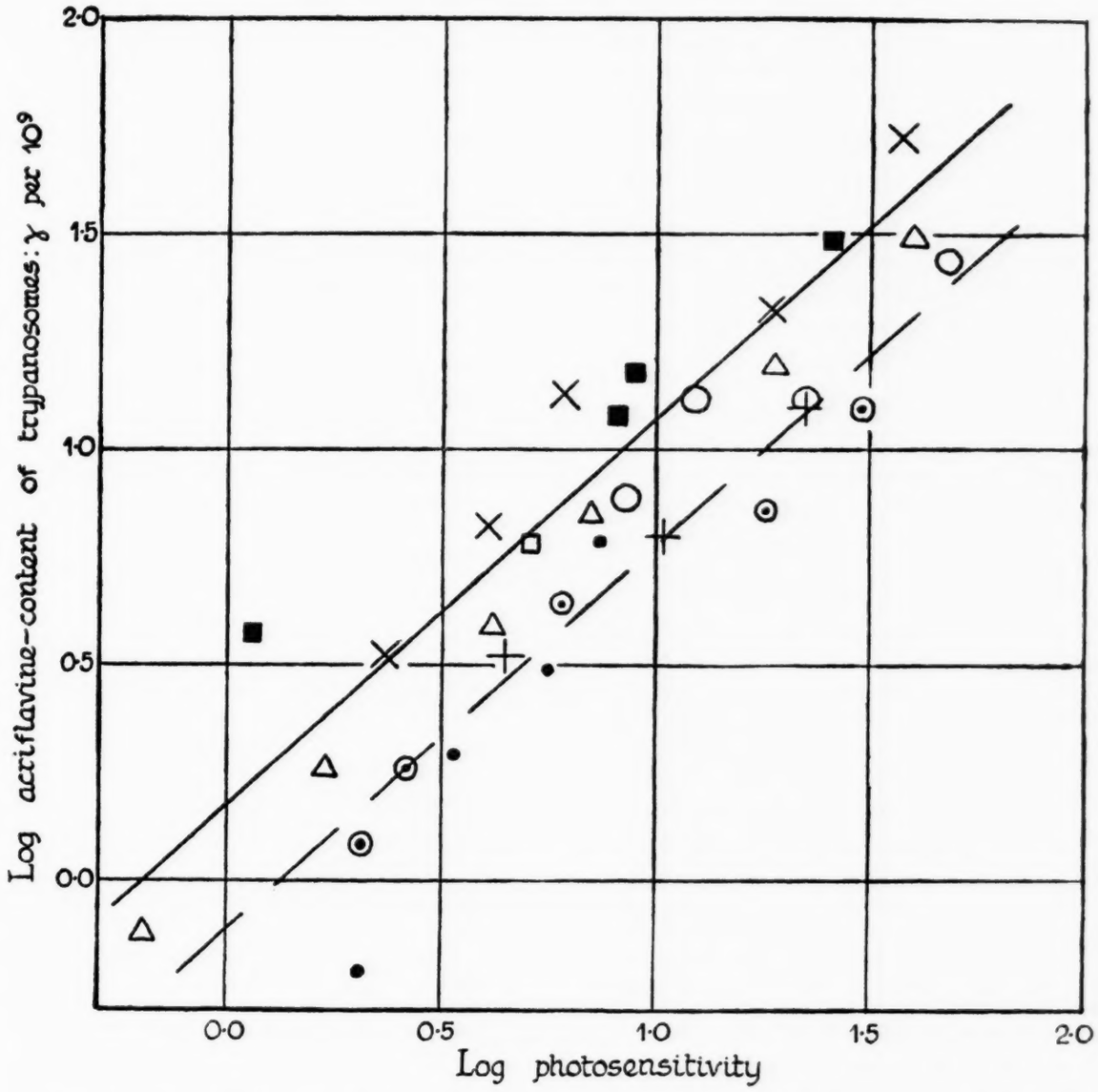


FIG. 4. Showing the relation between the acriflavine-content of the trypanosomes and their photosensitivity.

Temperature, 16–20° C.

The circles, spotted circles and spots represent three experiments with resistant trypanosomes, and the other points were obtained with normal ones. The continuous line is the graph for the normal trypanosomes, and the broken line that for the resistant ones.

On theoretical grounds, it is probable that the two curves are really identical; but for practical purposes they will be considered as distinct.

The main value of these curves is empirical; they enable the acriflavine-content of trypanosomes to be determined by measurement of their photosensitivity, which is easy to perform and which can be applied to small numbers of organisms. It is assumed that the photosensitivity is wholly due to the acriflavine in, or on, the parasite, and not to that present in the medium. Since the concentration in the trypanosome is much greater than that in the medium

(Hawking, 1938), this assumption seems justified ; in any case, when equilibrium has been reached there is a simple relationship between the two.

If the equations relating the photosensitivity to the acriflavine-content of the trypanosomes are correlated with those previously given for the photosensitivity and the initial concentration in the medium, it is possible to derive various other relationships, and to compare the values thus obtained with those based on direct measurement (see Hawking, 1938, fig. 6). In this way, the accuracy of the methods can be checked and their limitations explored.

Thus, for normal trypanosomes :

$$0{\cdot}86 \log C = \log P + 3{\cdot}972$$
$$1{\cdot}12 \log A = \log P + 0{\cdot}211$$

$\therefore A = 97{\cdot}7\ C^{10{\cdot}77}$ and y (the intracellular concentration, γ per ml.) = $4{,}024\ C^{0{\cdot}77}$. When the trypanosomes number 1,500 per mm.^{3},the total amount absorbed by the trypanosomes in 1 ml. is $0{\cdot}147\ C^{0{\cdot}77}\gamma$. With initial concentrations of 1γ, $0{\cdot}1\gamma$ and $0{\cdot}01\gamma$ per ml. the proportion absorbed by the trypanosomes is 15, 25 and 43 per cent. respectively. The partition-ratio is $\frac{4{,}024}{C^{0{\cdot}23} - 0{\cdot}147}$ and, for initial concentrations of 1γ, $0{\cdot}1\gamma$ and $0{\cdot}01\gamma$ per ml., the value for the ratio is 4,700, 9,200 and 20,000 respectively.

For initial concentrations of about $0{\cdot}1\gamma$ per ml. these results are in fair agreement with those previously determined experimentally, but on either side of this concentration the two sets of figures diverge. From a previous paper (Hawking, 1938, fig. 6), it seems clear that in the lower concentrations there is a simple direct proportion between the amount of acriflavine inside the trypanosome and the amount outside it, and that the partition-ratio is a constant. The value calculated from the photosensitivity, according to which the ratio varies approximately in inverse proportion to $C^{0{\cdot}23}$, is consequently incorrect, and indicates some fallacy in the underlying assumptions. The most probable fallacies are : (1) The assumption that the points of fig. 3 lie on a straight line (although experimentally this seems to be approximately the case), and the determination of the slope of this line, and similarly in fig. 4. (2) The omission of any reference to threshold concentration and threshold time in the calculation of the equations for photosensitivity. Similar calculations from the equations for resistant trypanosomes yield that the partition-ratio equals $\frac{371}{C^{0{\cdot}36} - 0{\cdot}0133}$, so that for initial concentrations of 1γ and $0{\cdot}1\gamma$ per ml. the partition-ratio is 383 and 870 respectively. These values are much higher than that determined experimentally, viz., 61, but the lower one is similar to that calculated from the experiment recorded by Pedlow and Reiner (1935), and would be more concordant with the measurements of the minimum trypanocidal concentration (Hawking, 1938) than the experimental value is. Perhaps future investigations will explain this discrepancy.

PHOTOSENSITIVITY OF TRYPANOSOMES EXPOSED *IN VIVO*

To compare the results of the present paper with those described by Jancsó, infected mice were injected subcutaneously with acriflavine, using doses and solutions similar to those used by him, and the photosensitivity of the trypanosomes was determined. The results are shown in Table III. Jancsó

TABLE III

Showing the photosensitivity of trypanosomes exposed to acriflavine *in vivo*

Mouse	Infection: parasites per field (1/6 obj. 4 oc.)	Dose of acriflavine per 20 gm. mouse	Time-interval: minutes	Average light-index: seconds	Corresponding concentration of acriflavine in blood: γ per ml.	Infection 24 hours later
1	20	0·05 mgm. (0·5 ml. 1/10,000)	45	41·2	0·010	++
2	20	„ „ „	50	38·4	0·011	++
3	4	0·1 mgm. (0·5 ml. 1/5,000	40	19·9	0·025	
4	5	„ „ „	60	8·6	0·069	
5	5	0·2 mgm. (0·2 ml. 1/1,000)	40	51·5	0·0078	2
6	7	„ „ „	45	3·9	0·19	2
7	10	0·4 mgm. (0·4 ml. 1/1,000)	45	3·8	0·20	1/20*
8	1/3	„ „ „	60	2·1	0·40	1/20*

*The blood of these mice became free from trypanosomes 48 hours after treatment.

(1931) gives the following results:

Dose 0·05 mgm. per 20 gm., light-index 45 sec. at 35 min. after injection, and 84 sec. at 65 min.

Dose 0·1 mgm., light-index 51 and 60 sec. respectively.

Dose 0·2 mgm., light-index 32 and 37 sec. respectively.

Dose 0·4 mgm., light-index 14 and 32 sec. respectively.

Dose 0·5 mgm., light-index 11 and 28 sec. respectively. The minimum effective dose for his strain, which just sufficed to clear the blood of parasites in 48 hours, was 0·4 mgm., similar to the dose required for the present strain. The figures for the light-index recorded in Table III are somewhat irregular, apparently owing to variations in the individual mice, but taking the average it would seem that the illumination used in the present case was much stronger than that used by Jancsó, and that the light-indices recorded by him are about four times as long as those obtained in the present series for the same dose of

acriflavine. As a matter of interest, the concentrations of acriflavine corresponding to these light-indices, obtained by reference to fig. 3, have been included in the table. No allowance has been made for the fact that the concentration recorded in fig. 3 is the *initial* concentration in the medium and not the *final* concentration, which is in equilibrium with the trypanosomes; to allow for this a correction of about 20 per cent. should be added.

It is desirable that future workers with this technique should include in their determinations a series which can be compared with those obtained in other laboratories, so as to render possible reciprocal calibration of the respective optical systems. The easiest figures to obtain are those showing the photosensitivity at various initial concentrations in the medium (as in fig. 3); but the relation of the photosensitivity to the acriflavine-content of the trypanosomes (as in fig. 4) is more satisfactory theoretically.

DISCUSSION

The purpose of this paper has been to establish the quantitative relations between the photosensitivity of trypanosomes and the amount of acriflavine present in the organisms or in the surrounding fluid, so that the photosensitivity can be used as a convenient means of measuring these two latter quantities. Its use to estimate the acriflavine-content of trypanosomes, while exposed to repeated washings, has been illustrated elsewhere (Hawking, 1938, Table IV), quantities as small as $0{\cdot}001\gamma$ in a million trypanosomes being detected in this way. This method was also used for the determination of low concentrations of acriflavine in biological fluids, and its possibilities in this connection are further illustrated by the following experiment, in which attention is directed to the technique rather than to the intrinsic value of the results.

Experiment

A rabbit weighing 780 gm. was injected intravenously with 1·5 ml. of a 1/1,000 solution of acriflavine. At various intervals, blood was collected from the other ear by means of a blood pipette, diluted with 2 per cent. citrate, and mixed with a suspension of trypanosomes, so that the final number of trypanosomes was about 2,000 per mm.^{3} The tubes were kept at room-temperature in the dark for one hour, and the photosensitivity of the trypanosomes was determined in the usual way. From this, the concentration of acriflavine in the blood was obtained by reference to fig. 3. The chief data can be summarized thus:

Time after injection	7	14	30	60	minutes.
Dilution of blood	50	50	4	4	
Photosensitivity of trypanosomes (100/light-index) ..	5·1	2·0	3·9	2·9	
Concentration of acriflavine in blood	1·3	0·40	0·072	$0{\cdot}052\gamma$	per ml.

The initial concentration of acriflavine in the blood (calculated) was 38γ per ml. The rapidity with which the dye leaves the blood is remarkable, but similar results have been obtained with other compounds by many other observers.

The question was considered whether this technique could be applied to the determination of other fluorescent substances of medical interest, but the results were disappointing. Atebrin musonate will make trypanosomes photosensitive *in vitro*, but relatively high concentrations, $0{\cdot}3$–10γ per ml., are

required, and large changes of concentration produce only small changes in the photosensitivity ; these experiments are described in more detail in a subsequent paper. Apparently atebrin is not readily absorbed by trypanosomes. Plasmoquine and quinine produced no photosensitivity. Jadin (1932) found that eosin and erythrosin, 1/100,000 *in vitro*, rendered trypanosomes photosensitive, but did not do so *in vivo* ; fluorescin was inactive. Fischl and Singer (1934) found no sensitivity after exposing trypanosomes (? *in vivo*) to eosin, erythrosin or various other compounds. Jancsó (1932) states that photosensitivity can be induced *in vivo* by Browning's compound 2-(p-acetylamino-styryl) 6-dimethylaminoquinolinmethosulphate. Probably this technique can be applied only to substances which are both fluorescent and readily absorbed by trypanosomes.

SUMMARY

1. When trypanosomes are exposed to acriflavine *in vitro* or *in vivo*, they become photosensitive, owing to their absorption of the compound. The degree of photosensitivity can be measured quantitatively on the stage of a dark-ground microscope as the period of illumination required to cause immobilization.

2. Equations are given for (1) the relation between the photosensitivity and the initial concentration of acriflavine in the medium, and for (2) the relation between the photosensitivity and the acriflavine-content of the trypanosomes. These equations enable the photosensitivity to be used to measure low concentrations of acriflavine (0·2–0·001 γ per ml.), or to indicate the acriflavine-content of small numbers of trypanosomes ; the accuracy is about 30 per cent.

3. The resistant strain of trypanosomes shows a smaller range of individual variation than the normal one, the coefficient of variation in the resistant strain being 22 per cent. and in the normal strain 33 per cent.

Acknowledgements.—This work was assisted by a grant from the Medical Research Council. The atebrin musonate and plasmoquine were kindly supplied by Bayer Products, Ltd.

REFERENCES

Fischl, V., and Singer, E. (1934). Die Wirkungsweise chemotherapeutisch verwendeter Farbstoffe. *Ztschr. f. Hyg. u. Infekt.*, **116**, 348.

Hausmann, W., and Krumpel, O. (1929). Ueber Absorption von Leukoverbindungen und Farbstoffen in ihrer Beziehung zur photobiologischen Sensibilisation im Ultraviolett. *Wien. Klin. Woch.*, **42**, 546.

Hawking, F. (1938). Analysis of the trypanocidal action of trivalent arsenicals and acriflavine. *Ann. Trop. Med. & Parasitol.*, **32**, 313.

Jadin, J. (1932). La photo-sensibilité de quelques parasites. *C. R. Soc. Biol.*, **110**, 124.

Jancsó, N. von (1931). Photobiologische Studien in der Chemotherapie. I : Lichtempfindliche Trypanosomen in trypaflavinbehandelten Tieren. *Zentralbl. f. Bakt., Orig.*, **122**, 388.

——— (1932). Mechanismus der Arzneifestigkeit bei Protozoen : zur Frage der Parasitotropie chemotherapeutischer Mittel. *Ibid.*, **124**, 167.

Oesterlin, M. (1936). Zur Chemotherapie der Infektionskrankheiten. *Ztschr. f. Hyg. u. Infekt.*, **118**, 263.

Pedlow, J. T., and Reiner, L. (1935). On the mechanism of chemotherapeutic action. XII : Comparison of the binding of chemotherapeutic agents by normal and resistant trypanosomes. *Jl. Pharmacol.*, **55**, 179.

A NOTE ON THE TRYPANOCIDAL ACTION OF ATEBRIN IN RELATION TO ITS ABSORPTION BY TRYPANOSOMES

BY

FRANK HAWKING*

(From the Department of Pharmacology, Welsh National School of Medicine, Cardiff)

(Received for publication July 26th, 1938)

In 1934 Fischl and Singer described *in vivo* experiments to show that atebrin was absorbed by trypanosomes but exerted no trypanocidal action; from which it was concluded that, although the absorption of a chemotherapeutic agent is a necessary preliminary to its parasiticidal action, it is not identical with it. Oesterlin (1936) also refers to atebrin as being trypanocidally completely inactive, and yet readily absorbed by trypanosomes. The object of the present communication is briefly to demonstrate that, although admittedly the absorption of some chemical compounds may occur without chemotherapeutic effect, yet this is not the case with atebrin, since its slight tendency to be absorbed is in approximate proportion to its small trypanocidal activity.

The methods and the strains of trypanosomes (*T. rhodesiense*) used were the same as those of the previous papers (Hawking, 1938*a*, 1938*b*). The preparation of atebrin used was atebrin musonate, kindly supplied by Bayer Products, Ltd.

Trypanocidal action. The trypanocidal action of atebrin was examined *in vitro* by the Liverpool technique (see Yorke and Murgatroyd, 1930). Under these conditions at 37° C. the minimum trypanocidal concentration for normal trypanosomes after 6 hours' exposure was 5γ per ml., and after 24 hours' exposure 1·25–2·5γ per ml. For atoxyl-resistant trypanosomes the results were the same or slightly less. The corresponding figures for acriflavine are 0·33 and 0·039γ per ml. for normal trypanosomes, and 5 and 0·63 γ per ml. for resistant ones. Thus, *in vitro* the trypanocidal action of atebrin on normal trypanosomes is about $^1/_{32}$ of that of acriflavine, and on resistant trypanosomes it is about one half. Examined *in vivo*, the maximum tolerated dose of atebrin, 5 mgm. per 20 gm. mouse intraperitoneally, had practically no trypanocidal action on normal parasites, a result which confirms the findings of Fischl and Singer. (In a later paper Singer (1935) admits that atebrin is trypanocidal *in vitro*, but he gives no details of the concentration required, and he attaches no significance to the observation, since he considers that the chemotherapeutic behaviour of trypanosomes *in vitro* is quite different from that *in vivo*.)

Absorption. The absorption of atebrin *in vitro* was examined in a similar way to that used for acriflavine in a previous paper (Hawking, 1938*a*). A heavy

*Gillson Scholar of the Society of Apothecaries of London.

A NOTE ON THE TRYPANOCIDAL ACTION OF ATEBRIN IN RELATION TO ITS ABSORPTION BY TRYPANOSOMES

BY

FRANK HAWKING*

(*From the Department of Pharmacology, Welsh National School of Medicine, Cardiff*)

(*Received for publication July 26th*, 1938)

In 1934 Fischl and Singer described *in vivo* experiments to show that atebrin was absorbed by trypanosomes but exerted no trypanocidal action; from which it was concluded that, although the absorption of a chemotherapeutic agent is a necessary preliminary to its parasiticidal action, it is not identical with it. Oesterlin (1936) also refers to atebrin as being trypanocidally completely inactive, and yet readily absorbed by trypanosomes. The object of the present communication is briefly to demonstrate that, although admittedly the absorption of some chemical compounds may occur without chemotherapeutic effect, yet this is not the case with atebrin, since its slight tendency to be absorbed is in approximate proportion to its small trypanocidal activity.

The methods and the strains of trypanosomes (*T. rhodesiense*) used were the same as those of the previous papers (Hawking, 1938*a*, 1938*b*). The preparation of atebrin used was atebrin musonate, kindly supplied by Bayer Products, Ltd.

Trypanocidal action. The trypanocidal action of atebrin was examined *in vitro* by the Liverpool technique (see Yorke and Murgatroyd, 1930). Under these conditions at 37° C. the minimum trypanocidal concentration for normal trypanosomes after 6 hours' exposure was 5γ per ml., and after 24 hours' exposure 1·25–2·5γ per ml. For atoxyl-resistant trypanosomes the results were the same or slightly less. The corresponding figures for acriflavine are 0·33 and 0·039γ per ml. for normal trypanosomes, and 5 and 0·63 γ per ml. for resistant ones. Thus, *in vitro* the trypanocidal action of atebrin on normal trypanosomes is about $^1/_{32}$ of that of acriflavine, and on resistant trypanosomes it is about one half. Examined *in vivo*, the maximum tolerated dose of atebrin, 5 mgm. per 20 gm. mouse intraperitoneally, had practically no trypanocidal action on normal parasites, a result which confirms the findings of Fischl and Singer. (In a later paper Singer (1935) admits that atebrin is trypanocidal *in vitro*, but he gives no details of the concentration required, and he attaches no significance to the observation, since he considers that the chemotherapeutic behaviour of trypanosomes *in vitro* is quite different from that *in vivo*.)

Absorption. The absorption of atebrin *in vitro* was examined in a similar way to that used for acriflavine in a previous paper (Hawking, 1938*a*). A heavy

*Gillson Scholar of the Society of Apothecaries of London.

suspension of normal trypanosomes in equal parts of serum and Locke's solution was mixed with the compound dissolved in an equal volume of Locke's solution. After 20 minutes' exposure at room-temperature the trypanosomes were centrifuged off without washing, and their content of drug was investigated by extraction with alcohol and comparison of the colour of the extract with known concentrations. The concentration in the supernatant fluid was determined in a colorimeter. The protocol of an experiment is shown in Table I, which

TABLE I

Showing the absorption of atebrin and diamino-methyl-acridine by normal trypanosomes

Tube	Drug	Initial concentration of drug : γ per ml.	Total drug extracted from trypanosomes : γ	Concentration of drug found in supernatant fluid : γ per ml.	Partition-ratio
1	Atebrin	80	Trace (?8)	About 80	? 7
2		20	0	,, 20	0
3	Diamino-methyl-acridine	40	140	1·5	6,900

Volume per tube, 4 ml. Temperature, 23° C. Duration of exposure, 20 minutes.
No. of trypanosomes per tube, $6{\cdot}2 \times 10^8$. Volume of trypanosomes per tube, 15 mm.^{3}
All trypanosomes were motile at the end of the exposure.

includes a parallel experiment with 2–8 diamino-10 : methyl-acridine for comparison. Acriflavine is the commercial preparation of diamino-methyl-acridine, diluted with 20–40 per cent. diamino-acridine. The deposit of trypanosomes from tube 1 was very pale yellow, and that from tube 2 was white ; that from tube 3 was dark orange, and microscopically the trypanosomes were intravitally stained yellow. (This agrees with the observation of Fischl and Singer, that the deposit of trypanosomes, exposed to acriflavine *in vivo*, was yellow, while that exposed to atebrin was white ; they could detect the presence of atebrin in the organisms by means of the fluorescent microscope.) Table I shows that the trypanosomes have only a slight tendency to absorb atebrin. The amounts found in the deposits were too small for accurate determination. The partition-ratio of the drug between trypanosomes and medium, i.e., concentration in trypanosomes/concentration in medium, as calculated in a previous paper (Hawking, 1938*a*), was about 0–30 for various experiments with atebrin, while with diamino-methyl-acridine it was about 7,000. The contrast is clear. The partition-ratio of acriflavine for resistant trypanosomes is about 60.

Photosensitivity. Solutions of atebrin are feebly fluorescent. Acriflavine, which is strongly fluorescent, is able to render trypanosomes photosensitive, so that, when exposed to strong light on the stage of a dark-ground-illumination microscope, they rapidly become immobile and presumably dead. Atebrin

was investigated from this aspect, using the technique recently described (Hawking, 1938*b*), with which the present data are directly comparable. Table II shows the result of two experiments on different days with normal

TABLE II

Showing the photosensitivity produced in trypanosomes by exposure to atebrin *in vitro*

Concentration of atebrin : γ per ml.	Average light-index	
	Normal trypanosomes	Atoxyl-resistant trypanosomes
20	—	31
10	26	—
5	29	48
1·25	27	77
0·31	40	? 145
0·16	128	·150

Temperature, 37° C. Trypanosomes, about 1,500 per mm.^{3}

Light-index = Period of illumination required to immobilize trypanosomes, in seconds.

and resistant trypanosomes respectively. Atebrin produces definite photosensitivity, but its power in this respect is low compared with that of acriflavine —e.g., normal trypanosomes, exposed to acriflavine 0·04 γ per ml., have a light-index of 19 seconds ; and resistant ones, exposed to 0·16 γ per ml., have a light-index of 21 seconds. The relation between the concentration of drug and the degree of photosensitivity induced is much less close in the case of atebrin than it is in the case of acriflavine. When infected mice were treated intraperitoneally with the maximum tolerated dose of atebrin (5 mgm. per 20 gm.) and blood was withdrawn from the tail half an hour later, the trypanosomes showed only very slight photosensitivity, an exposure to light of 4–6 minutes being required to immobilize them ; if the blood was withdrawn after 2½ hours, no photosensitivity could be detected. Fischl and Singer found no photosensitivity after exposure to atebrin (apparently *in vivo*) ; but Oesterlin observed it when the trypanosomes were illuminated with ultra-violet light.

Conclusion. The evidence of these experiments indicates that *in vitro* atebrin is probably absorbed by trypanosomes, but that the amount taken up is small. Its trypanocidal power *in vitro* is much lower than that of acriflavine, but not lower than might have been expected from its smaller tendency to be absorbed. The partition-ratio of acriflavine for normal trypanosomes is about 8,000, that of atebrin is probably under 20, giving a proportion of 400 : 1 between them ; the proportion between their trypanocidal activities *in vitro* is about 32 : 1. Both absorption and trypanocidal action are quantitative, not absolute, terms; most substances will be absorbed, even if only to a minute extent, and most substances can kill trypanosomes if sufficiently concentrated. The comparison

should be made between the extent of the absorption and the degree of the trypanocidal activity.

The relation between atebrin and normal trypanosomes is much more similar to that between acriflavine and resistant trypanosomes. Thus the minimum trypanocidal concentration of atebrin for 6 hours' exposure is about 5γ per ml., and the partition-ratio is probably under 20; the minimum trypanocidal concentration of acriflavine for resistant organisms is 5 γ per ml., and the partition-ratio is 60; in neither case is the trypanocidal power great enough to be detected *in vivo*. On this basis, atebrin should be regarded, not, as Fischl and Singer suggest, as a substance which is absorbed by trypanosomes but has no trypanocidal power, but rather as one which is absorbed to such a small extent that its (potential) trypanocidal power can be exerted only in high concentrations. The evidence of the experiments *in vivo* are inconclusive. Presumably the toxicity of atebrin prevents the maintenance in the blood of sufficiently high concentrations during sufficiently long periods for the trypanocidal power to be manifested.

Although it is here denied that atebrin is an example of a compound which is absorbed by trypanosomes without exerting a trypanocidal action, it must be emphasized that such compounds, e.g., intravital strains, almost certainly do exist, and that combination between drug and trypanosome is not identical with trypanocidal action, although it is a necessary preliminary to it.

SUMMARY

Atebrin has been described by other workers as a compound which is absorbed by trypanosomes but exerts no trypanocidal action. Quantitative experiments *in vitro* show that it possesses a moderate trypanocidal activity which is as high as might be expected from its low tendency to be absorbed. Its behaviour towards normal trypanosomes is similar to that of acriflavine towards resistant ones.

ACKNOWLEDGEMENTS.—This work was assisted by a grant from the Medical Research Council. Acknowledgement is due to Bayer Products, Ltd., for the supply of atebrin musonate.

REFERENCES

FISCHL, V., and SINGER, E. (1934). Die Wirkungsweise chemotherapeutisch verwendeter Farbstoffe. *Ztschr. f. Hyg., u. Infekt.*, **116**, 348.

HAWKING, F. (1938*a*). Analysis of the trypanocidal action of trivalent arsenicals and acriflavine. *Ann. Trop. Med. & Parasitol.*, **32**, 313.

——— (1938*b*). A quantitative study of the photosensitivity induced in trypanosomes by acriflavine. *Ibid.*, **32**, 367.

OESTERLIN, M. (1936). Zur Chemotherapie der Infektionskrankheiten. *Ztschr. f. Hyg. u. Infekt.*, **118**, 263.

SINGER, E. (1935). Die Wirkungsweise der Chemotherapeutika bei Spirochäten- und Protozoeninfektionen. *Med. Klin.*, **31**, 386.

YORKE, W., and MURGATROYD, F. (1930). Studies in chemotherapy. III: The action *in vitro* of certain arsenical and antimonial compounds on *T. rhodesiense* and on atoxyl- and acriflavine-resistant strains of this parasite. *Ann. Trop. Med. & Parasitol.*, **24**, 449.

ON A COLLECTION OF ANOPHELINE MOSQUITOES FROM THE ISLAND OF HAINAN

BY

CH'I HO*

(*From the Department of Entomology, Liverpool School of Tropical Medicine*)

(*Received for publication October 26th*, 1938)

During a tour made in the island of Hainan by the writer in 1934, in connection with the Hainan Biological Expedition organized by four Chinese scientific research institutes, a careful collection of anopheline mosquitoes was made in all the places visited. Malaria is very prevalent in this island, and it was thought desirable that information regarding the possible vectors of the disease should be obtained and recorded.

The following species and varieties were taken during the tour :

Subgenus *Anopheles* Meigen

Group *Anopheles* Root

Series *Anopheles* Edwards

A. sintonoides Ho

Series *Myzorhynchus* Edwards

A. hyrcanus var. *sinensis* Wiedemann

A. hyrcanus var. *nigerrimus* Giles

A. barbirostris Van der Wulp

Subgenus *Myzomyia* Blanchard

Group *Neomyzomyia* Christophers

A. kochi Dönitz

Group *Myzomyia* Christophers

A. minimus Theobald

A. jeyporiensis var. *candidiensis* Koidzumi

Group *Pseudomyzomyia* Christophers

A. subpictus var. *indefinitus* Ludlow (?)

A. vagus Dönitz

Group *Neocellia* Christophers

A. maculatus var. *hanabusai* Yamada

A. philippinensis Ludlow

A. sintonoides was proved to be new to science and has recently been described by the writer (Ho, 1938). *A. hyrcanus* var. *sinensis*, *A. hyrcanus* var.

---

* Research Fellow of the China Foundation for the Promotion of Education and Culture.

nigerrimus, *A. barbirostris*, *A. minimus*, *A. jeyporiensis* var. *candidiensis* and *A. vagus* have been reported previously by Riley (1932*b*), and the four remaining species are now recorded from Hainan for the first time. The writer has not so far encountered the other three species of Riley's record, namely, *A. jamesii* Theo., *A. maculipalpis* Giles and *A. tessellatus* Theo.

Anopheles hyrcanus var. **nigerrimus** Giles, 1900

In the mainland of China *A. hyrcanus*, so far as is known, is represented only by var. *sinensis* ; in the island of Hainan, however, the darker Oriental form, var. *nigerrimus*, also occurs. The two varieties are extremely difficult to distinguish from one another, as the characters used to separate them are all more or less variable. The following table gives the results of an examination based upon 39 female var. *sinensis* and 3 female *nigerrimus*.

	A. hyrcanus var. *nigerrimus*	*A. hyrcanus* var. *sinensis*
General coloration	Dark.	Not so dark.
Subcostal spot	Small ; incompletely involving R_1 and fleck-like in all the 3 specimens.	Large ; often involving R_1 equally with the costa, but may be small and fleck-like, as in var. *nigerrimus*.
Hind tarsal banding	Broader ; the base of 4th joint, and in one specimen the base of 5th joint also, pale.	Narrow ; apices of joints only pale.
Stems of R_{2+3} and M	Dark.	Whitish.
Vein Cu	Dark area at base long.	Dark area at base short.
Vein An	The two dark areas longer, with a short intervening white area.	The two dark areas short, separated by a long pale area.
Fringe-spot at Cu_2	Absent in all 3 specimens.	Present in 12, doubtful in 3, absent in 24.

This variety has been recorded from Hainan by Riley (1932*b*).

BREEDING-PLACE. The three female specimens were bred out from larvae taken in a rice-field.

Anopheles hyrcanus var. **sinensis** Wiedemann, 1828

BREEDING-PLACES. In Hainan the larvae of this variety have been taken in ponds, rice-fields, flooded grass-fields, drainage channels and streams with grassy edges.

Anopheles barbirostris Van der Wulp, 1884

ADULT. FEMALE. A large black anopheline. Length of wing 3·5–4·0 mm.

Head. Palpi very shaggy, entirely without pale markings; palpal index 0·80. Labium shaggy on basal half or basal two-thirds.

Thorax. Anterior pronotum with a dense tuft of outstanding scales. Propleural hairs 5, associated with some pale scales. Spiracular, prealar and upper mesepimeral hairs numerous. Mesonotum dark, sparsely covered with curved, pale, hair-like scales and chaetae. Anterior promontory with lateral dense tufts of dark scales.

Wings. Prominently speckled, owing to mixture of broad dark and pale scales on most veins. Basal half of costa with scattered pale scales. Subcostal pale spot small; preapical pale spot small, involving R_1 and R_2. Apex of wing

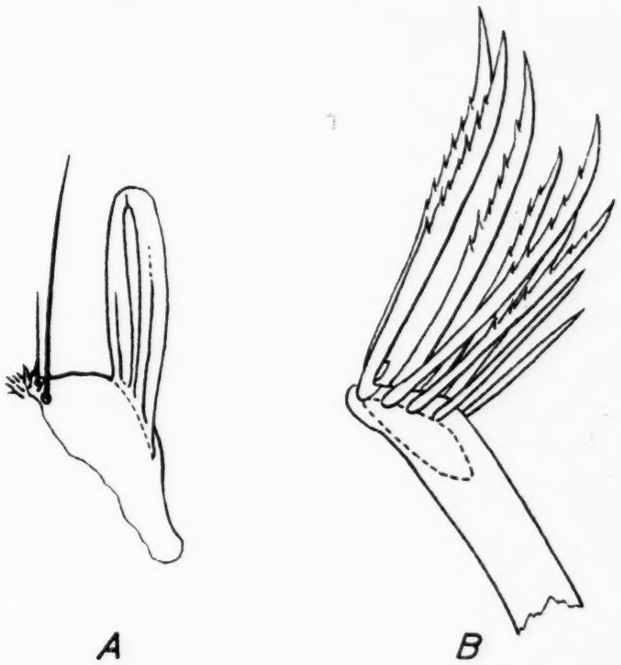


FIG. 1. *A. barbirostris* Van der Wulp. Male terminalia. *A.*—Harpago; *B.*—Phallosome.

dark, without a broad apical pale fringe-area as is found in *A. hyrcanus*. R_3 with two small pale spots. R_{4+5} with pale and dark scales about equal in number, tending to form batches. A fringe-spot at the end of R_{4+5}. Vein M mainly dark, apical third with scattered pale scales. Cu speckled, fringe-spot at Cu_2 absent. Vein An speckled on basal half, the distal half with two dark spots. Humeral vein with a few dark scales.

Legs. Tibiae narrowly pale at base and apex. Fore tarsus with narrow, terminal, pale bands on joints 1 and 2. Mid tarsus entirely unbanded. Hind tarsus with narrow, pale, apical bands on 1–4.

Abdomen. Dorsum dark, devoid of scales, except for a few narrow pale ones on segment VIII. Venter with scattered broad pale scales. Segment VII with a large tuft of black scales apically in the middle line.

MALE. *Terminalia.* Fig. 1. Two parabasal spines and an internal spine about half-way down coxite. Harpago with a broad flattened club and two slender spines, the longer of which is about the length of the club. Phallosome long, slightly curved in lateral view; leaflets 4 or 5, long, strongly chitinized, markedly serrated, measuring 98μ, 94μ, 74μ and 46μ respectively. Ninth tergite with rather short processes, which are not so long as interval between.

PUPA. Fig. 2. *Paddle.* Index 1·4. Paddle hair short, straight, bifid; accessory hair finer, about same length as paddle hair, bifid.

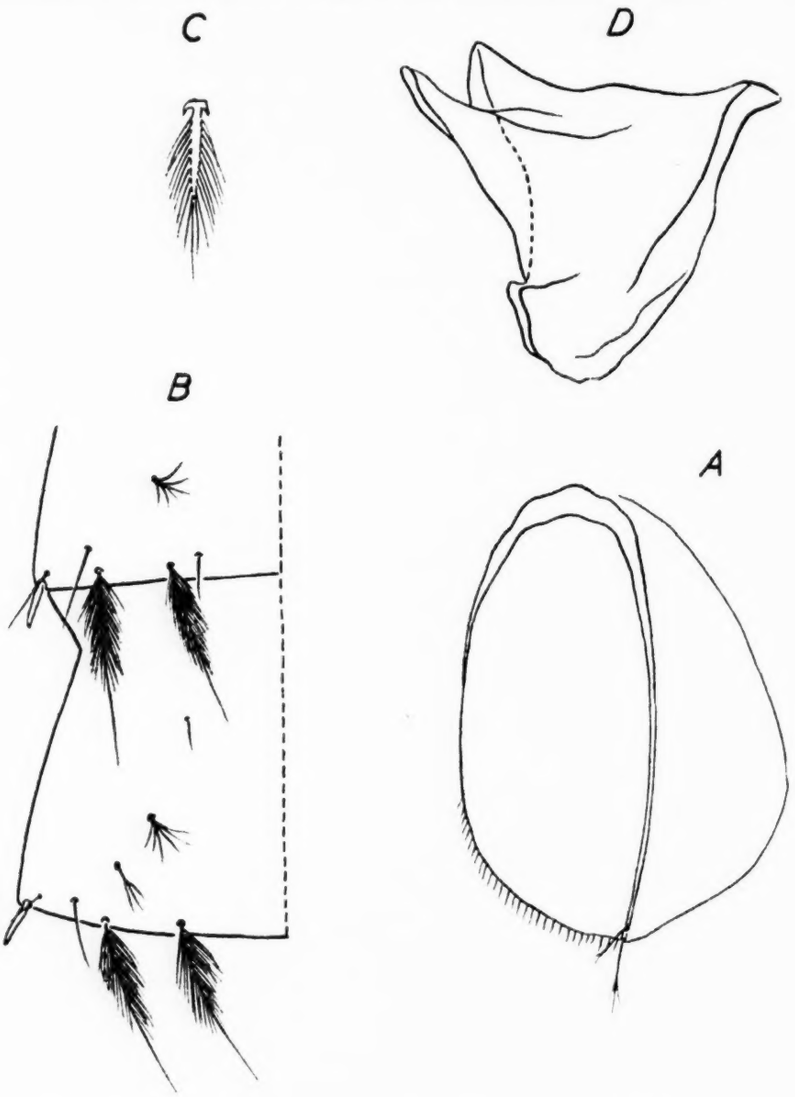


FIG. 2. *A. barbirostris* Van der Wulp. Pupal details. *A.*—Paddle; *B.*—Abdominal segments VI–VII; *C.*—Spine of segment VIII; *D.*—Trumpet.

Spine. VIII, short, thick, with fine branches. II–VII, short, blunt, thick, reduced in size on more anterior segments.

Hairs B and C. III–VII, tufted, darkly pigmented, with the median hair of each especially long, extending about half the length of the segment.

Other hairs very short; hair 4 many-branched, hair 5 simple or bifid. Hairs 1–3 mostly simple, may be 3- or 4-branched.

The respiratory trumpets large, deeply cleft.

BREEDING-PLACES. This species is rare in Hainan. A single larva was first found among larvae collected from a rice-field containing standing water and much vegetation. Another larva was taken from a flooded grassy field in association with *A. minimus* and *A. philippinensis*. A third was also taken in a flooded grassy field. Altogether one male and two female adults were bred.

Anopheles kochi Dönitz, 1901

ADULT. FEMALE. A medium-sized, light-coloured anopheline. Length of wing 3·07 mm. (average of 8 females).

Head. Palpi ornamented with 4 white, 3 yellow, and 5 narrow black bands, the black bands at base of each segment. Labium yellow in apical half and with some spots of yellow on the basal black portion, extreme tip of labium sometimes dark.

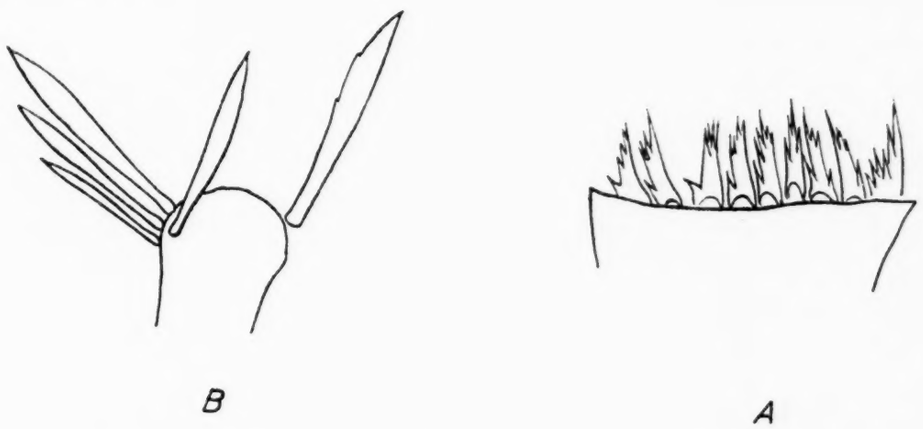


FIG. 3. *A. kochi* Dönitz. *A.*—Pharyngeal armature; *B.*—Phallosome.

Pharynx. Fig. 3, *A*. Teeth in a single row, about 9 in number, not developed as rods and cones, with fimbriated extremities.

Thorax. Mesonotum slightly pinkish in ground-colour, with a dark eye-spot on each side of dorsum and a less conspicuous one at the base of each wing. Scutellum also with a dark spot in the median lobe.

Wings. Extensively pale, the dark areas very much reduced in size. Base of wing sometimes with two well-separated dark spots instead of three, as figured by Christophers (1933). Subcostal and preapical pale spots vary considerably in length. Vein M extensively dark on the portion near the fork. Otherwise the wing-marking is the same as shown in Christophers' figure.

Legs. Femora and tibiae speckled. First joint of fore and mid tarsi and first and second joints of hind tarsus speckled laterally. Fore tarsus with apical and basal banding at all joints except last, usually with anterior aspect mainly

affected. Mid tarsus with inconspicuous apical bands. Hind tarsus with first joint narrowly banded apically, second with broad apical band, third and fourth with broad apical and basal bands, fifth with apical half of joint white.

Abdomen. Dorsum with yellow scales on II–VII ; venter with a prominent tuft of black scales on the distal end of each segment from II–VII.

MALE. *Terminalia*. Fig. 3, *B*. Instead of 6 leaflets, as described by Christophers, there are only 4 on each side, 3 of which are about the same size, the fourth one smaller ; they measure 35μ, 33μ, 28μ and 22μ respectively, the longest showing some serrations.

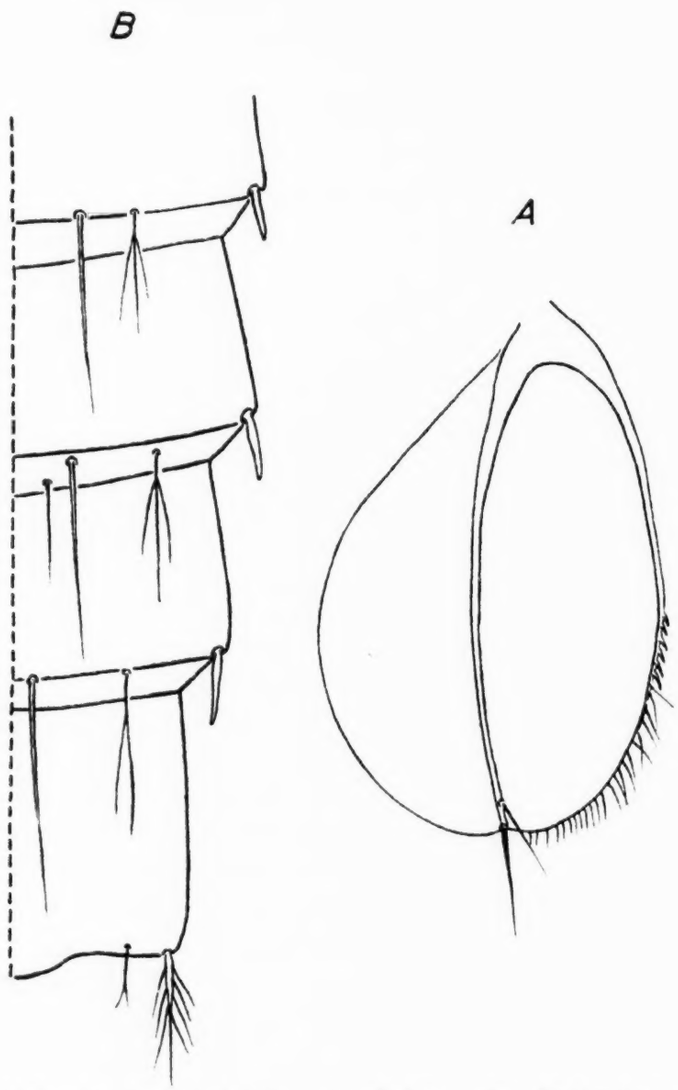


FIG. 4. *A. kochi* Dönitz. Pupal details. *A*.—Paddle ; *B*.—Abdominal segments V-VIII.

PUPA. Fig. 4. *Paddle*. The basal half of the external border smooth, the apical half with fine sharp spines, varying from a few to about 15, which are abruptly replaced by fine hairs on the posterior border. Paddle hair short, straight, simple ; accessory hair short, bifid.

Spine. VIII, 1/2 length of segment, 8–10 branched ; accessory hair short, bifid. VII, less than 1/4 length of segment, rather sharp-pointed. II–VI, progressively shorter, somewhat blunt.

Hair B. V-VII, bifid or trifid, about 2/3 length of segment. IV, 4-branched ; III, many-branched ; both short.

Hair C. V–VII, simple, about 2/3 length of segment. IV, trifid. III, 4-branched. *Hair C* of VI simple.

All other hairs short, mostly simple ; *hair no*. 4, 2–4 branched.

This species has once been recorded from China (Canton) by Buddle (1928).

Breeding-Places. Larvae were taken on one occasion from a small pool with a muddy bottom, and on another occasion from a flooded grassy field, together with larvae of *A. maculatus* var. *hanabusai* and *A. philippinensis*. Three male and six female adults were bred.

Anopheles minimus Theobald, 1901

Female. *Head*. Palpal banding shows a great deal of variation even in this short series of 14 females (fig. 5, *A*). In one extreme case the short intervening dark band is entirely wanting ; in other cases it varies from being very short to about twice as broad as the subapical white band. In more typical specimens the dark band is shorter than either of the white bands, but in three specimens it is considerably broader than the subapical white, approaching a condition similar to that of *fluviatilis*, from which these three specimens differ in having an incomplete interruption on the base of the costa. The presence of such a palpus in *minimus* had already been noted by Evans (1930, p. 591) from Hongkong specimens. Proboscis shows no pale tache on apical half in any of the specimens.

Pharynx. Filaments, as described by Christophers (1933), narrow, thorn-like, without fimbriated ends and lateral spicules ; lateral teeth and crest spines both conspicuous (fig. 5, *C*). The hair-like processes of the post-armature ridges very short, the length of the longest being less than half the width of one ridge (fig. 5, *D*).

Wings. Basal third of costa tending to show two different forms of ornamentation : in one the pale interruption is well marked, in the other it is incomplete. It is incomplete in the three specimens with *fluviatilis*-like palpi and in a fourth one, taken with them on the same date, which has a slightly shorter dark intervening band on palpi. Without further knowledge of their breeding-habit and the characters of the corresponding male, it is perhaps still unjustifiable to say that they belong to a distinct variety. The dark costal area at the extreme base is further interrupted in some specimens by a small patch of a few pale scales at a point a little beyond the humeral cross-vein. On the apical half of the wing the pale and dark areas measure, on an average, as follows : subcostal pale area 0·19 mm., preapical dark spot 0·46 mm., preapical pale area 0·16 mm., and

apical dark spot 0·21 mm., of which the preapical dark and preapical pale are the most variable, the former varying from 0·23 to 0·50 mm., the latter from 0·10 to 0·20 mm. R_{4+5} mainly pale, with small dark area at or near the two ends. In one case the pale area on its middle portion is reduced to less than 1/3 the length of the vein. Vein Cu_1 with a central white spot in 9 specimens and without it

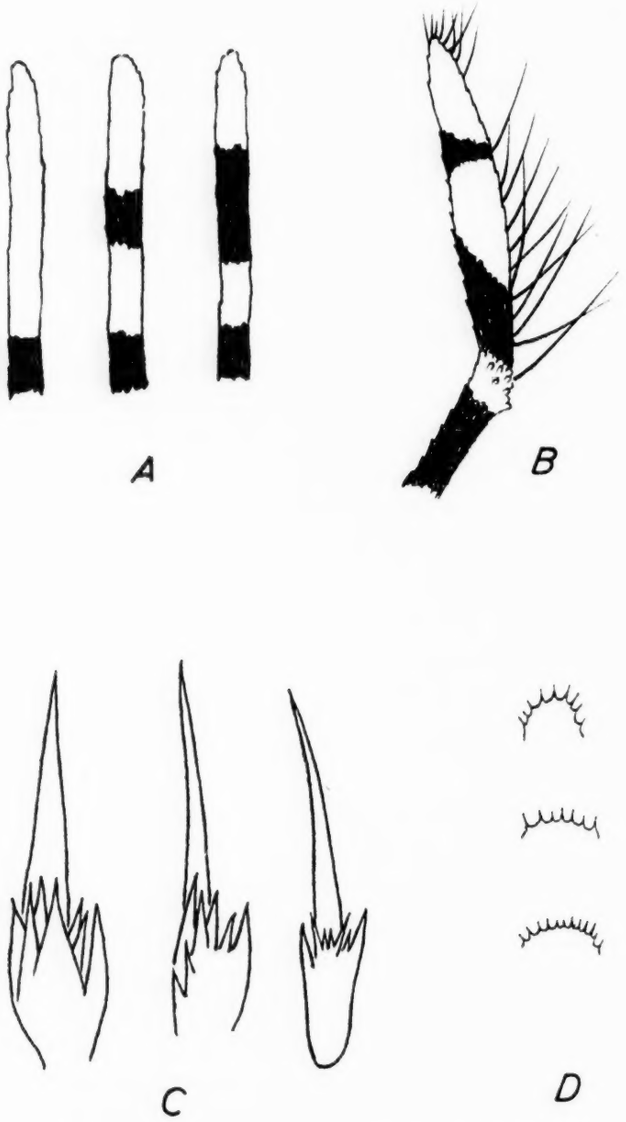


FIG. 5. *A. minimus* Theo. *A.*—Female palpus, showing variation in amount of white on apical and penultimate segments; *B.*—Male palpus; *C.*—Pharyngeal teeth; *D.*—Post-armature ridges.

in 5 specimens. In the latter case the branch is extensively dark, the pale area at the cross-vein being also reduced. Vein M_1 more often without pale area. Vein An commonly has a long dark spot on the distal half and a short one on the basal half, with a short white area between the two; in two specimens the

vein is entirely dark, and in one specimen it has three dark spots. Fringe-spot at An is absent in all the specimens. Wing length, on an average, 2·6 mm.

Legs. Fore and mid tarsi of all specimens showing rather distinct indication of narrow banding.

MALE. Palpi ornamented with 3 pale spots on club (fig. 5, *B*).

Terminalia. Harpago with large stout apical hair considerably longer than the club, and an external accessory hair of about the same length as the club. Inner accessory hair usually absent ; two short delicate ones present in one case (fig. 6, *A*). Phallosome with 4 leaflets on each side, all markedly serrated, the longest measuring 36μ (fig. 6, *B*).

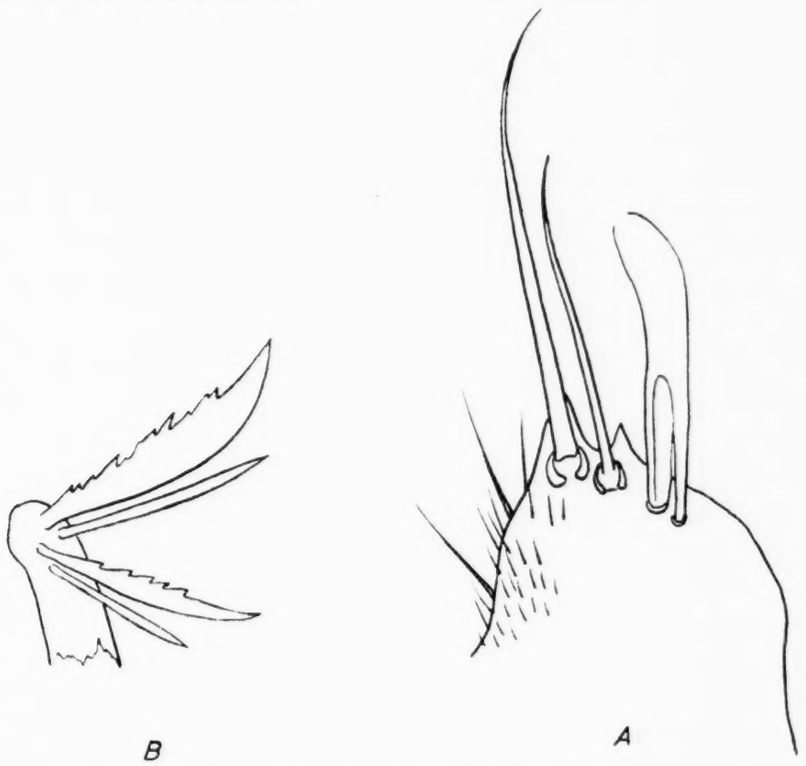


FIG. 6. *A. minimus* Theo. *A.*—Harpago ; *B.*—Phallosome.

The known distribution of *A. minimus* in China has been shown in Feng's map (1935) to be in the provinces of Yunnan, Kwantung, Fukien, Chekiang, Kiangsi and the island of Hainan. The Yangtsi River seems to be the northern limit of its distribution. The same author (1932) and Jackson (1934), working at Amoy and Hongkong respectively, both reported the highest rate of natural infection for this species. Evidently it is the chief vector of malaria in China.

In Hainan this species and *A. vagus* are the only anophelines which have been found in human dwellings, *A. vagus* being invariably more abundant than *A. minimus*. In hilly regions they were found to be the predominant species of the house-frequenting mosquitoes, the two species combined representing over 90 per cent. of the total capture ; in the plains, however, several culicine species

predominate, *A. minimus* and *A. vagus* together making up only about 20 per cent. of the total capture.

Anopheles jeyporiensis var. **candidiensis** Koidzumi, 1924

One male and one female of this variety are represented in the collection.

FEMALE. *Head.* The single female showed the relative lengths of subapical white, subapical black and apical white of palpi as 2 : 3 : 7 (fig. 7, *A*).

Thorax. Mesothoracic scaling, as in type-species, confined to the median area of the dorsum.

Wings. Wing-marking showing slight differences from the type, as follows: pale interruption on R_1 in preapical dark spot situated towards the outer end of the spot ; scaling on R_1 in median dark costal spot less than half the length of that on costa, and placed on the outer side of the spot ; vein R_{4+5} extensively pale, its dark area very short ; vein An pale on basal half, with two dark spots on apical half. Fore, mid and hind tarsi all ornamented as in the type.

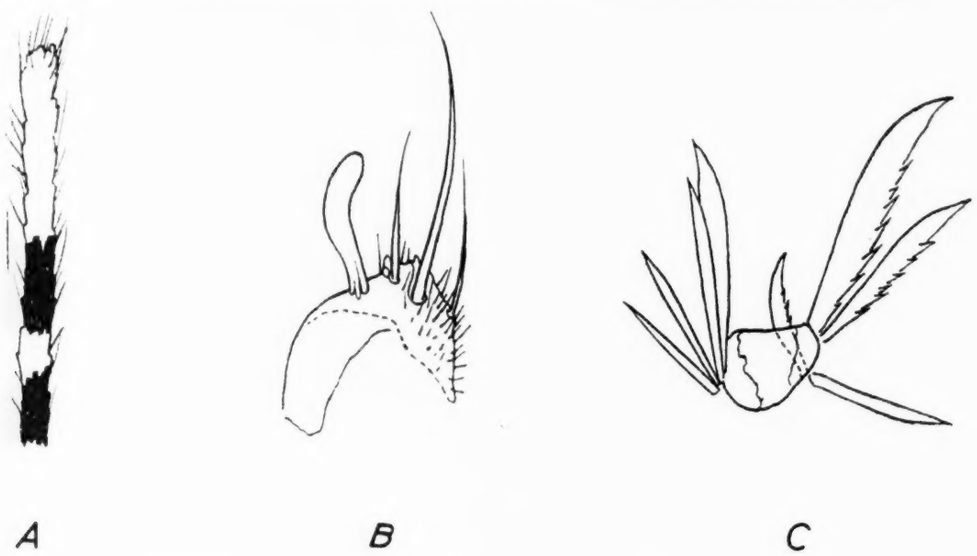


FIG. 7. *A. jeyporiensis* var. *candidiensis* Koid. *A.*—Female palpus ; *B.*—Harpago ; *C.*—Phallosome.

MALE. The single male found differs from the female in the following respects : median dark costal spot with a distinct accessory pale area ; preapical dark costal spot with the pale interruption on R_1 situated towards inner end of the spot ; vein R_{4+5} with longer dark area; veins R_{2+3} and Cu more extensively dark ; vein An with a dark spot on basal half, continuously dark on apical half ; mid tarsi unornamented on all the segments.

Terminalia. Fig. 7, *B*, *C*. Harpago, as in the type-form, with a longish hair between club and apical hair. Leaflets only 4 on each side, in place of 5–6 as described for the type-form ; shape and serrations on leaflets as usual for the *Myzomyia* group ; lengths of leaflets 32μ, 26μ, 20μ and 16μ.

In China this form has been recorded from Hongkong (Edwards, 1915; Jackson, 1934), Canton (Wu, 1936), Yunnan (Gaschen, 1934), Fookien (Feng, 1932; Lee, 1932) and Hainan (Riley, 1932*b*). Although some of these records gave the name of the type-form for its identification, they probably all referred to var. *candidiensis*.

Anopheles subpictus var. **indefinitus** Ludlow (?)

FEMALE. The single female found showed the essential characters, as described in *A. vagus*, to be as follows: (1) Length of subapical dark band of palpi 3/4 that of apical white band; this ratio falls outside the range of variation for both *vagus* and *vagus* var. *limosus*. (2) Labium without a lighter tache towards distal end. (3) Prehumeral accessory dark costal spot continuous and without white scales on anterior margin. (4) Subapical costal dark spot obviously longer than white spots on either side, occupying 40 per cent. of the entire length of the three spots. (5) Dark area on R_1 on inner costal spot just half the length of that on costa. (6) White interruptions on basal third of costa, one on one side, two on the other. (7) Fringe-spot between veins Cu_2 and An absent. (8) Hind tarsi with narrow, apical, white banding on segments 1–4, without basal banding.

The palpal banding and the absence of the tache on the labium serve to differentiate the specimen from the *vagus* series, from which they are otherwise indistinguishable. Whether or not there is a tache on the labium of female *subpictus* var. *indefinitus* is not mentioned by King, nor by Yamada in his description of *formosaensis II*, which is considered by King to be synonymous with var. *indefinitus*. This fact strongly suggests that the tache is most probably absent in var. *indefinitus*. If that be the case, the combination of the two characters would justify its being identified as var. *indefinitus*. But unfortunately the larval pelt of this specimen had been lost, and a verification from larval characters could not, therefore, be made.

Edwards (1915) recorded *A. rossi* var. *indefinitus* in the list of mosquitoes by Macfarlane from Hongkong. His *A. rossi* var. *indefinitus* refers to *vagus* (synonymy of Christophers, 1916, p. 477). Buddle (1928) and Faust (1926) recorded *A. rossi* from Kowloon and Canton respectively, but neither gave characters for their identification. Riley (1932*a*) identified a single specimen in Howard's collection captured near Canton as *A. subpictus*, which, as was alluded to later (Riley and Wu, 1932, p. 185), must be a specimen with *subpictus*-like palpi, i.e., a broad, subapical, dark band. Since true *subpictus* does not extend so far east, these records most probably refer to var. *indefinitus* or even to *vagus*, as has been stated by Christophers.

At present, since there is no account of the characters of the Canton and Hongkong specimens, and since there are no other available specimens, or early stages for confirmation in the present specimen, the records of var. *indefinitus* in all these three places cannot be considered as authentically established.

Anopheles vagus Dönitz, 1902

ADULT. FEMALE. *Head.* Subapical dark band on female palpi narrow, averaging only slightly over 1/4 the length of apical white, that of *vagus* var. *limosus* and *subpictus* var. *indefinitus*, according to King (1932), being about 1/3 and 1/2 of their corresponding apical white. The average measurements of 24 females are as follows :

Average length of apical white band ..	0·32 mm.
Average length of subapical dark band..	0·09 mm.
Average ratio of black to white ..	0·29
Range of variation in ratio	0·14–0·48

A rather faint lighter tache towards the distal end of the labium is present throughout the series, except in two, where its presence is doubtful ; in *vagus* var. *limosus* it is said by King to be entirely absent.

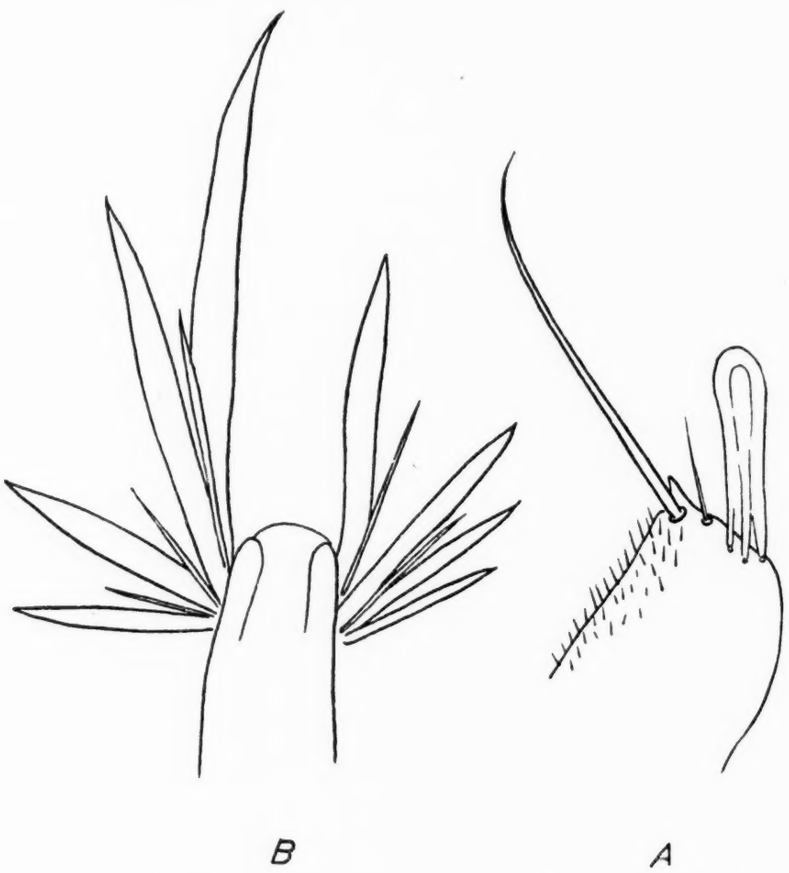


FIG. 8. *A. vagus* Dönitz. Male terminalia. *A.*—Harpago ; *B.*—Phallosome.

Wings. The prehumeral dark accessory costal spot on the extreme base of the wing, which has been found useful in distinguishing *subpictus* var. *indefinitus*, *vagus* and *vagus* var. *limosus*, was found in this series to be extremely variable and too indefinite to be of diagnostic value. Out of the 24 females, there were 11 with

this spot uninterruptedly dark, 7 with white scales on the anterior margin of the spot, 5 with white and dark scales mixed, and 1 with white scales only. The subapical costal dark spot, instead of being usually shorter, as described by Christophers (1933), is generally as long as or longer than the white spots on either side, its length being intermediate between that of *vagus* var. *limosus* and *subpictus* var. *indefinitus*. The average measurements of 24 females are as follows :

Average length of subapical white ..	0·32 mm.
Average length of subapical black ..	0·37 mm.
Average length of subcostal white ..	0·34 mm.
Percentage of black	36
Range of variation in percentage of black	28–49

Dark area on R_1 on inner costal spot often less than half the length of that on costa, averaging 41 per cent. of the whole area ; in one specimen it is entirely absent. Two white interruptions on basal third of costa formed by the humeral white spot and the presector white spot ; the former is always present, the latter may or may not be present. Fringe-spot between Cu_2 and An absent in all specimens except one.

Legs. Hind tarsi with narrow apical white banding on segments 1–4 and very often with basal pale scales on segments 4 and 5.

MALE. *Legs*. Fore tarsi with broad apical pale bands on segments 1 and 2 and usually with a narrower one on segment 3 ; in about a third of the total number of specimens, segment 3 also with some basal pale scales.

Terminalia. Fig. 8. Phallosome with four stout leaflets and a number of spikes on each side (fig. 8, *B*). Leaflets long, straight, with pointed ends, resembling a blade of grass in shape ; average length of the longest leaflets 69μ, that of the remaining three progressively shorter, being 49μ, 33μ and 23μ respectively. In three slide-preparations made of isolated phallosome, no serration was seen in any of the leaflets. Spikes about 4–7 in number, one or two of them almost attaining the length of the second leaflet, the rest short.

PUPA. *Paddle*. Fig. 9, *A*. The short denticles, present from near the base of the external border, becoming rather stoutish spines posteriorly and replaced abruptly by hairs at about the junction of anterior two-thirds and posterior third. The hairs decreasing in size and not quite reaching the paddle hair. Paddle hair long, hooked ; accessory hair simple or bifid (fig. 9, *B*).

Spine. VIII (fig. 9, *C*), 11–15 branched, somewhat more than 1/2 the length of the segment ; VI–VII, attenuated, about 2/3 the length of their segments ; V, similar, shorter, about 1/2 the length of the segment ; III–IV, blunt, short.

Hair B. IV–VIII, about 3/4 the length of the segment, 2–4 branched from near the base ; III, 4-5 branched.

Hair C. V–VIII, slender, simple, longer than segment ; IV, 3–4 branched, longest branch about reaching the posterior margin of segment V ; III, 4–6 branched, shorter.

Hair No. 4. III–VII, 3–4 branched.

LARVA. Fig. 10. Inner clypeal hairs slender, simple, 0·22 mm. in length, the distance between their bases slightly less than three times that between inner clypeal and outer clypeal of one side. Outer clypeal hairs simple, short, about 1/4 to 1/3 length of inner clypeal. Posterior clypeal hairs about the same size as outer clypeal, placed well forward on the clypeus, with their distal third extending beyond bases of inner clypeal. The distance between the two posterior clypeal hairs just half that between the two inner clypeal hairs. Inner sutural hairs slender, simple, slightly shorter than inner clypeal. Outer sutural hair 4-branched.

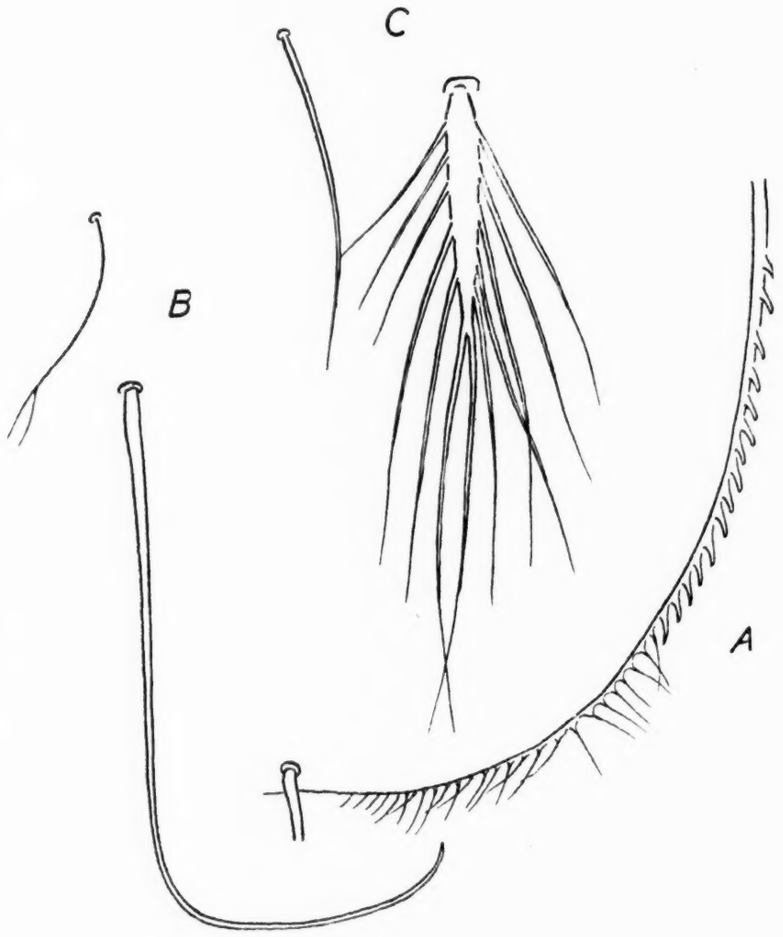


FIG. 9. *A. vagus* Dönitz. Pupal details. *A.*—Outer posterior border of paddle ; *B.*—Paddle and accessory hairs ; *C.*—Spine and accessory hair of segment VIII.

A series of 9 female and 12 male adults of *vagus* of Macfarlane's collection from Hongkong is in the possession of the Department of Entomology of the Liverpool School of Tropical Medicine. With the late Dr. Evans's kind permission, the writer had the opportunity of comparing them with the Hainan form. They show the following principal characters : apical white band of

female palpi distinctly more than twice the length of subapical dark band in all the specimens ; a light tache present in 6 of the 9 females, and rather doubtful in the remaining 3 ; in both sexes the subapical costal dark spot is as long as or longer than the white spots on either side—in only one specimen of each sex is it shorter ; prehumeral accessory dark costal spot with pale scales on anterior margin in all the females, continuously pale or with pale and dark scales mixed in males ; two white interruptions on basal third of costa in the majority of the specimens, single only in two males ; dark area on inner costal spot on R_1 about half the length of that on costa in female and tending to be a little longer in male ; leaflets of phallosome exactly like those of the Hainan form.

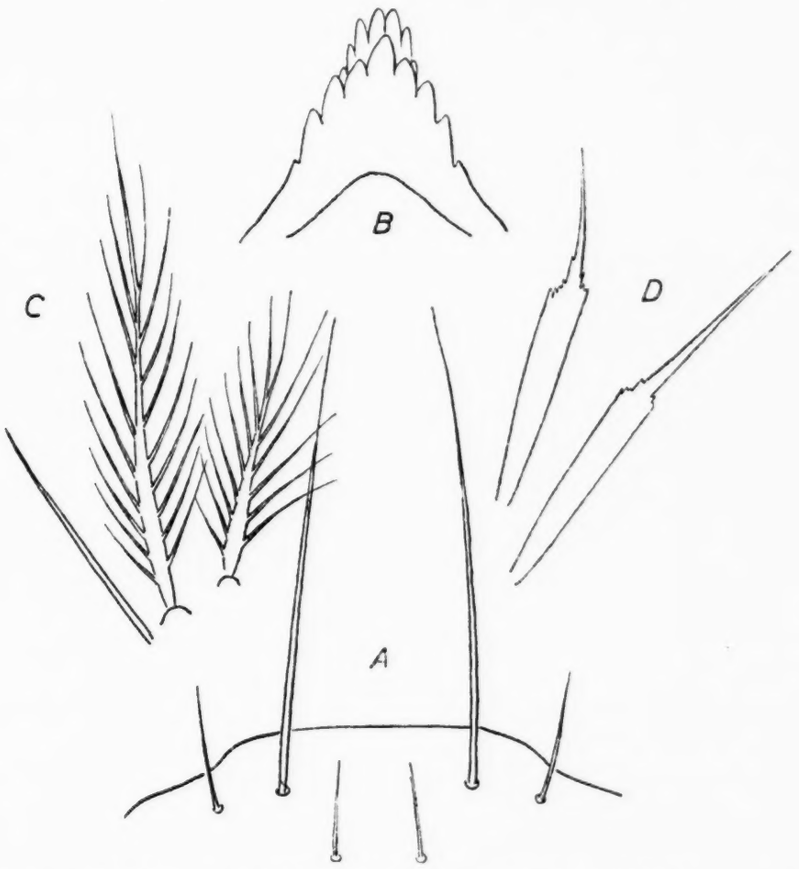


FIG. 10. *A. vagus* Dönitz. Larval details. *A.*—Clypeal hairs ; *B.*—Mentum and submentum ; *C.*—Shoulder hairs of the prothorax ; *D.*—Leaflets of palmate hair of segment IV.

In the main, therefore, *A. vagus* of Hainan and Hongkong agree pretty closely with Christophers' description. A few points of difference deserving special attention are the broader subapical dark costal spot, the rather indefinite character of the prehumeral accessory dark costal spot, and the shape, and perhaps also the number, of leaflets of the phallosome.

In Hainan, *A. vagus* have been recorded by Dr. Riley (1932*b*) from Nordoa and Kecheck. The present writer captured them in all the places he visited in the

island ; it is one of the commonest anophelines. As the records of his daily catches of indoor mosquitoes indicate, it also represents the most common house-frequenting anopheline. From March 26th to April 1st inclusive, among a total number of 78 anophelines caught indoors, 53 belong to this species, amounting to 68 per cent. Again, out of a total of 670 indoor mosquitoes (including *Anopheles*, *Culex* and *Aëdes*) caught from August 6th to 28th, 130 belong to this species, or about 20 per cent. No other species of *Anopheles* reached the same high percentage. A number of female *vagus*, which had by chance entered the mosquito-net covering the present writer's bed and were captured the next morning, were found all fully engorged with blood.

BREEDING-PLACES. Early stages were taken three times from rice-fields, and twice from flooded grassy ground. They were invariably found in association with the early stages of *maculatus* var. *hanabusai*, *philippinensis* and *hyrcanus* var. *sinensis* ; on one occasion they were found together with those of *kochi*.

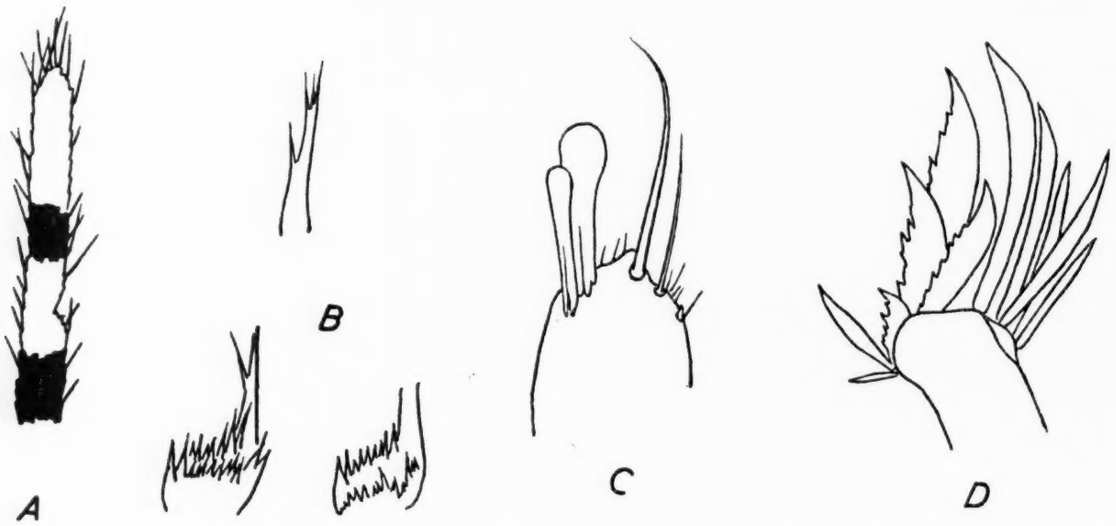


FIG. 11. *A. maculatus* var. *hanabusai* Yamada. *A*.—Female palpus ; *B*.—Pharyngeal teeth ; *C*.—Harpago ; *D*.—Phallosome.

Anopheles maculatus var. **hanabusai** Yamada, 1925

ADULT. FEMALE. Size rather small ; average length of wing 2·74 mm.

Head. The apical segment of palpi about half (or more) length of the penultimate segment (index 0·57). The average proportion of the apical pale band, the short intervening dark band and the subapical band is 4 : 1 : 3, which shows very little variation (fig. 11, *A*). Speckling of palpi not found in the series.

Pharynx. Fig. 11, *B*. Number of rods and cones about 10. Filaments of cones tapering, without pronounced lateral spicules ; crest with double row of rather regular spines. Rods with tips often bifid or trifid.

Thorax. Scales on mesonotum 7–10, striated ; those on the middle area mostly lanceolate or spindle-shaped, with pointed tips ; those on lateral area narrowly oval, with rounded tips.

Abdomen. Scaling on abdominal tergites closely resemble Yamada's description (Yamada, 1925) and Christophers' illustration (Christophers, 1933, fig. 46, 5*a*). Fairly numerous, narrow, spatulate, creamy scales sparsely distributed over the posterior half on segments II–IV or V, and rather densely over the whole surface of the remaining posterior tergites. Those scales on second tergite do not amount to a well-marked patch. Black scales present at the posterior angles of segments VI–VIII, and in two cases also on V, those on VII usually most pronounced.

Wings. Wing-markings similar to that figured by Christophers (1933, fig. 46, 1), having broad pale interruptions on apical half of costa. The length of the subapical dark spot not exceeding the combined length of the two pale spots on either side. The measurements of 9 specimens give the relative length of this dark spot to the combined length of the dark and pale spots as follows :

Average	43 per cent.
Range of variation . .	36–46 per cent.

Legs. Fore tarsus with broad apical and basal pale bands at 1–2, 2–3 and 3–4, base of fourth joint sometimes without pale scales. Mid tarsus with apical taches on first two or three joints. Hind tarsus with narrow apical pale band on first joint and broad ones on second to fourth joints, the fifth entirely white. Small pale spots are present on first tarsal joint of all the three pairs of legs, in addition to those on femora and tibiae, and one or two may also occasionally be found on the second tarsal joint of mid and hind legs. The relative length of second and third apical bands to the length of their corresponding joints is as follows :

1. Apical bands of the second hind tarsal joint :—

Average	23 per cent.
Range of variation . .	17–29 per cent.

2. Apical band of the third hind tarsal joint :—

Average	36 per cent.
Range of variation . .	30–42 per cent.

MALE. *Terminalia.* Fig. 11, *C*, *D*. Harpago as shown in figure ; a second smaller club shown in the same figure is probably an abnormal form. Phallosome with 6 pairs of blade-shaped leaflets, each with a small number of serrations ; the longest leaflet measures 43μ.

PUPA. Fig. 12. *Paddle* broadly oval in shape, not as elongated as shown in Senevet's figure (Senevet, 1931, fig. 20). Spines on posterior two-thirds of external border fine, pointed, giving place imperceptibly to hairs on posterior border ; the latter not extending beyond the paddle hair. Paddle hair long, hooked. Accessory hairs bifid or trifid.

Spine. VIII, about 1/2 length of its segment, having 7–10 branches on each side (fig. 12, *B*) ; V–VII, attenuated, about 1/2 length of succeeding segments ; IV, short and blunt (fig. 12, *C*).

LARVA. Fig. 13. Only two pelts were available for examination. Measurements of clypeal hairs of these two pelts : inner clypeal 0·16 mm., outer clypeal 0·08–0·11 mm., posterior clypeal 0·06 mm., all which are smaller than the figures given by Puri (1931). Posterior clypeal hair also differs from Puri's description in that its distal end reaches to just about the base of inner clypeal, not beyond it. Filament of palmate hairs of abdominal segments III–VII rather uniformly short, mostly about 1/4 of blade-length, rarely exceeding 1/3. All other larval characters agree closely with Puri's description.

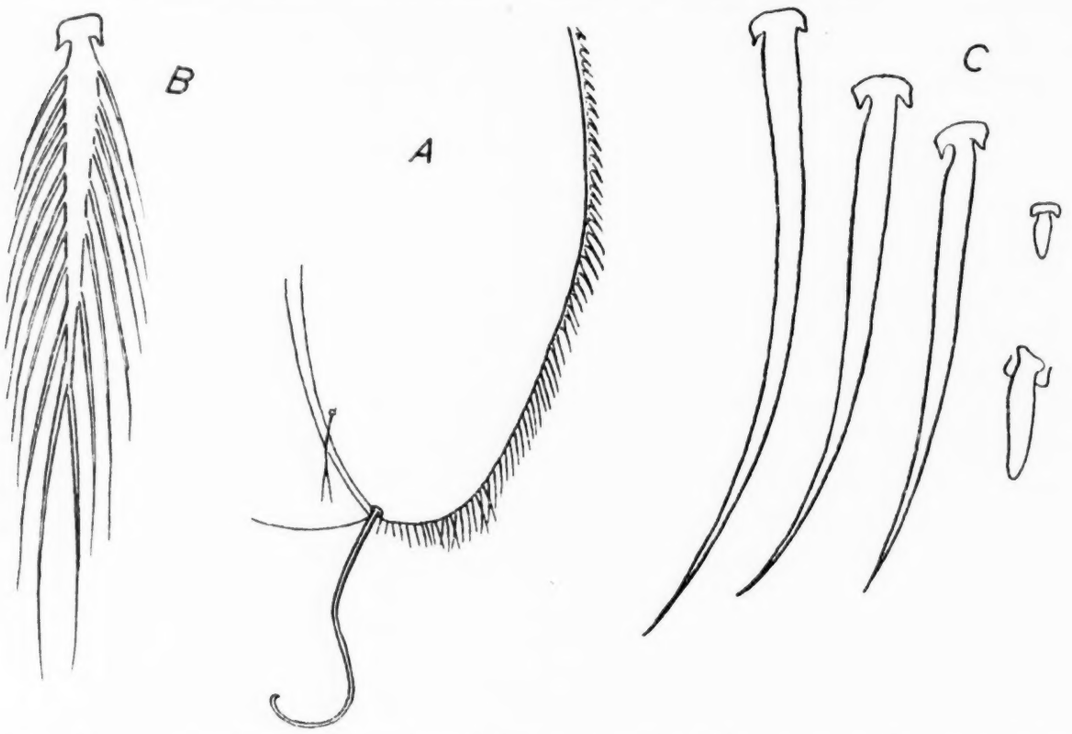


FIG. 12. *A. maculatus* var. *hanabusai* Yamada. Pupal details. *A*.—Paddle ; *B*.—Spine of segment VIII ; *C*.—Spines of segments III–VII.

From a careful comparison of the foregoing with Yamada's description, there can be no doubt that the Hainan and the Formosan forms are identical. The most important feature of them is the *willmori*-like scaling on the abdominal tergites, which differs from the type-form in being a constant character. The scaling differs from that of var. *willmori* in the fact that the scales are narrower and sparsely distributed over the anterior three or four tergites, and that those on the second tergite do not amount to a well-marked patch. Next in importance are the broad pale interruptions in the apical half of the wing and the broad tarsal banding. They are associated characters in Hainan and Formosan forms with quite a limited range of variation. In the type-form, as Christophers points

out, the pale interruptions are very variable in extent, and the usual length of the second and third apical band of the hind tarsus is 1/6 and 1/4 respectively of their joints ; whereas in var. *willmori* the pale interruptions are much narrowed, owing to the elongated preapical dark spot, but the hind tarsal banding is about the same as in the Hainan and Formosan forms.

From these differences it is quite obvious that the Hainan and Formosan forms together represent a quite distinct form from both the type-form and var. *willmori*, and the writer therefore proposes to rank it as a variety, *A. maculatus* var. *hanabusai* Yamada.

Series of *A. maculatus* collected from Hongkong by Macfarlane in 1914 and by Severn in 1916 are in the possession of the Department of Entomology of the Liverpool School of Tropical Medicine ; and the late Dr. Evans noted in Severn's collection a marked tendency to melanism in the wing-markings as well as in certain other characters. With Dr. Evans's permission, the present writer

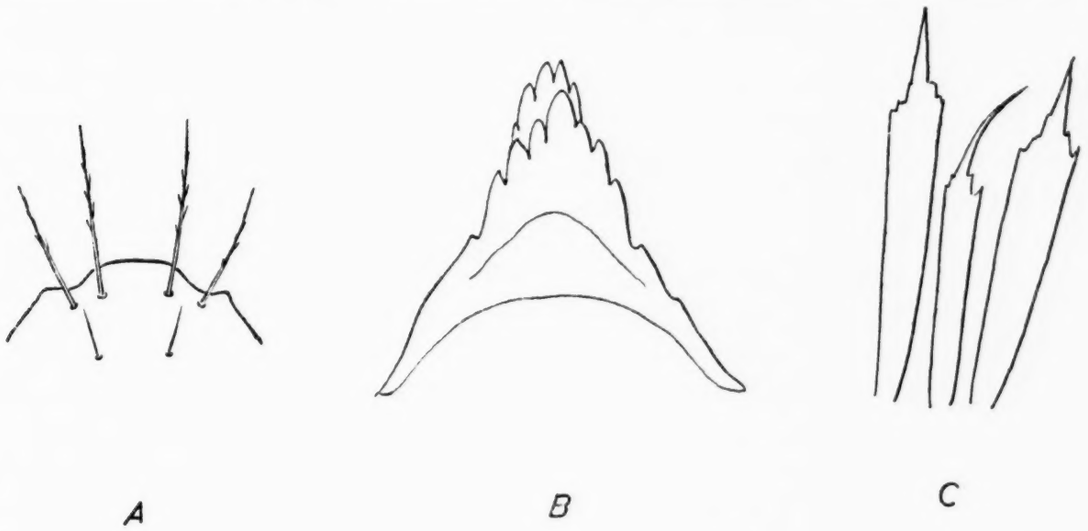


FIG. 13. *A. maculatus* var. *hanabusai* Yamada. Larval details. *A.*—Clypeal hairs ; *B.*—Mentum and submentum ; *C.*—Leaflets of palmate hair of segment V.

examined both collections in detail, and the results of these observations are as follows. The two series of specimens appear to be of two quite different forms. Those of Macfarlane's collection are smaller in size, with broader tarsal banding and with broader pale interruptions on the costa, all of which characters agree closely with the Hainan form. The abdominal scaling, however, is different, being confined to the last three tergites. There are very few, if any, scales on the tergites before VI, and those on these last three tergites are narrow, golden, heavily mixed with concolorous hairs, thus making them even more inconspicuous. In its general appearance, therefore, the abdomen closely resembles what Theobald has described as ' Abdomen black, with pale golden hairs which became very dense and bright golden at the apex of the abdomen ', though he does not mention the presence of any scales. It is obviously upon this form

that Theobald based his original description of the type-form. The specimens of Severn's collection, on the other hand, are invariably characterized by their larger size, darker appearance, narrower pale interruptions on the costa, and narrower tarsal banding (average percentage of the preapical dark spot 60; that of second and third apical band of hind tarsus 12 and 23). Though the abdominal scaling is also confined to the last three or four tergites the scales are broader.

Rather contrary to the writer's expectation for this Hongkong material are the facts that in neither series does the abdominal scaling equal that of the Hainan-Formosan form, and that, as the two series of specimens indicate, there are possibly more than one form of *maculatus* occurring at the type-locality itself. The writer is therefore inclined to think that what Christophers thought to be the very variable type-form is probably a mixture of two or more forms, and that a careful study of the specimens from the type-locality is urgently needed in order to clarify the rather unsatisfactory state of the species.

Breeding-Places. Of the six larval specimens collected, two came from rock-holes along a mountain stream, two from a grassy portion of flooded marshy land, one from the edge of a stream with algal growth and slow-running water, and one from a shallow spring-fed pool with a sandy bottom, in which no plants were growing. Nine female and two male adults were bred.

Anopheles philippinensis Ludlow, 1902

Adult. Female. A rather dark, small-sized species (average length of wing 2·54 mm.).

Head. Palpal index 0·64. Apical pale band about equal to its preceding dark area. Some dark scales are sometimes found on the apical pale band.

Pharynx. Number of cones and rods about 12. Filaments of cones rapidly tapering, usually not frayed, but sometimes frayed into 1 or 2; crest massive, with double row of rather fine, regular spines. Rods with two spines, one behind the other (fig. 14, *A*).

Thorax. Scales on mesonotum mostly 6–8 striated, with truncated tips. Some on its middle area narrower, longer, with pointed tips. Prothoracic lobes and pleurae of practically all the specimens show presence of scales, the rare minority without scales being probably denuded specimens.

Abdomen. On dorsum, scaling begins from segment V, in a few cases from IV. Scales dark-brownish; on V and VI they are few in number and confined to the posterior part of each segment, on segments VII and VIII they cover the entire segment. Pale scales only seen on last two segments. Scales on ventral side confined to segments VI–VIII, rarely also on V, dark and pale mixed.

Wings. Throughout the series of 30 specimens the subcostal pale area involves R_1, and the middle part of vein Cu is extensively pale without a dark spot in the region of origin of the branch. The relative amount of dark and pale scaling on the costa varies considerably. The measurements of the

30 female specimens give the relative length of the subapical dark spot to the combined length of the dark and pale spots as follows :

Average	.. ..	54 per cent.
Range of variation	..	38–62 per cent.

Legs. The femora and tibiae of all three pairs of legs and the first tarsal joint of the mid and hind legs usually with pale stripe on anterior side, that of

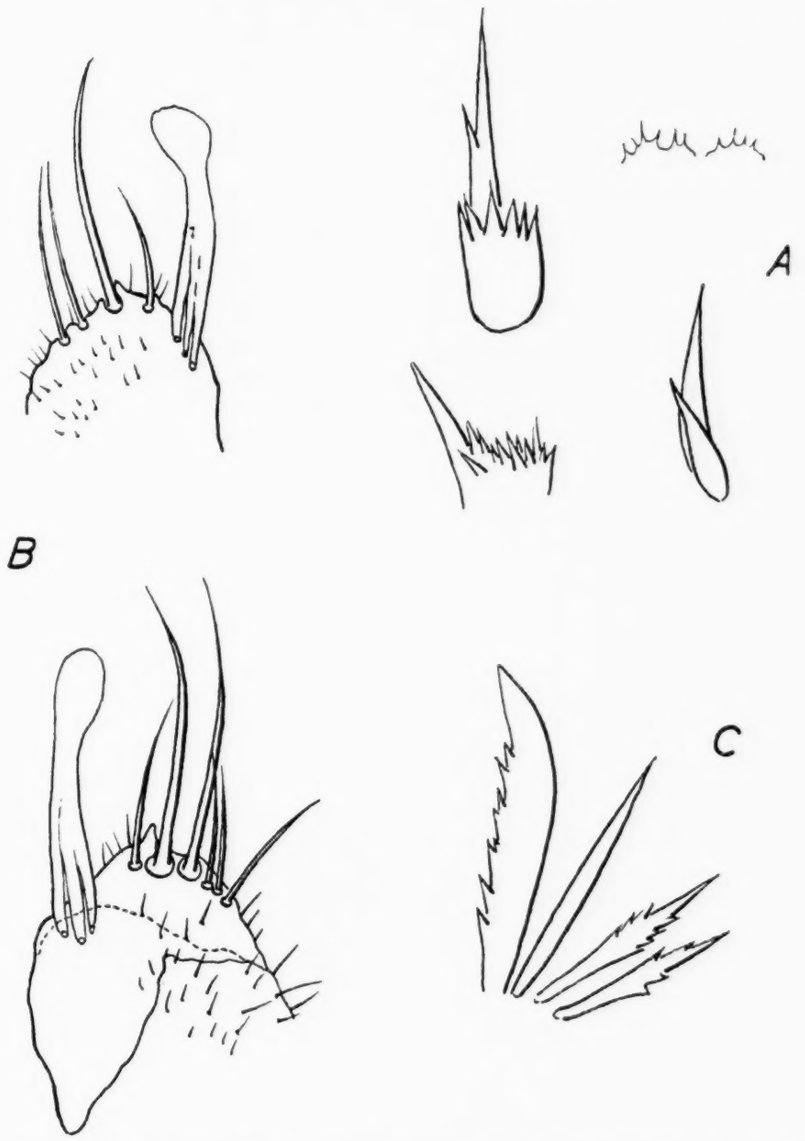


FIG. 14. *A. philippinensis* Ludlow. *A.*—Pharyngeal teeth and post-armature ridges ; *B.*—Harpagones ; *C.*—Leaflets of phallosome.

the mid leg being most pronounced. There is a conspicuous oval pale spot on the anterior surface towards the apex of the mid femora, and a rather ill-defined one in a similar position on the hind femora. Front tarsus with broad apical band on joints 1–3 ; mid tarsus with narrower apical band on joints 1–2 or 1–3.

The apex of the first hind tarsal joint in most cases picked out in some degree with white scaling ; in two or three of the most prominent ones the white scaling is in the form of a complete ring, about twice the diameter of the tarsal joint ; five of the specimens showed complete absence of this white interruption. The amount of white on the second hind tarsal joint varies considerably (average, 33 per cent. ; range of variation, 15–53 per cent.).

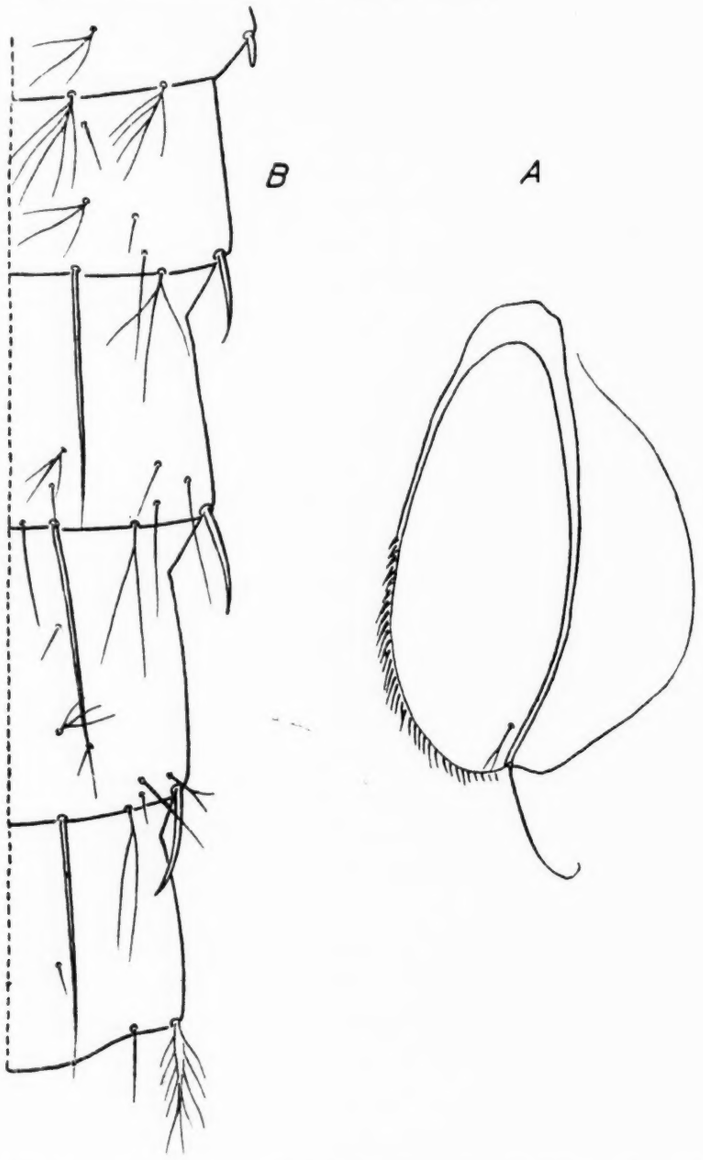


FIG. 15. *A. philippinensis* Ludlow. Pupal details. *A*.—Paddle ; *B*.—Abdominal segments IV–VIII.

MALE. *Terminalia*. Fig. 14, *B*, *C*. Harpago with apical spine about the same length as the club, a slightly shorter hair between it and the club, 1–3 about equally strong hairs on inner side of the harpago. Leaflets five on each side, blade-shaped, with well-marked serrations on one side, the two shortest leaflets having serrations on both sides. Length of longest leaflet 44μ, the

remaining four leaflets decreasing evenly in length as follows: 40μ, 32μ, 24μ and 20μ.

PUPA. *Paddle.* Fig. 15, *A.* Spines on posterior half of external border fine, pointed, and giving place imperceptibly to hairs on posterior border which do not extend beyond the paddle hair; paddle hair straight on basal two-thirds, hooked apically; accessory hair simple or bifid.

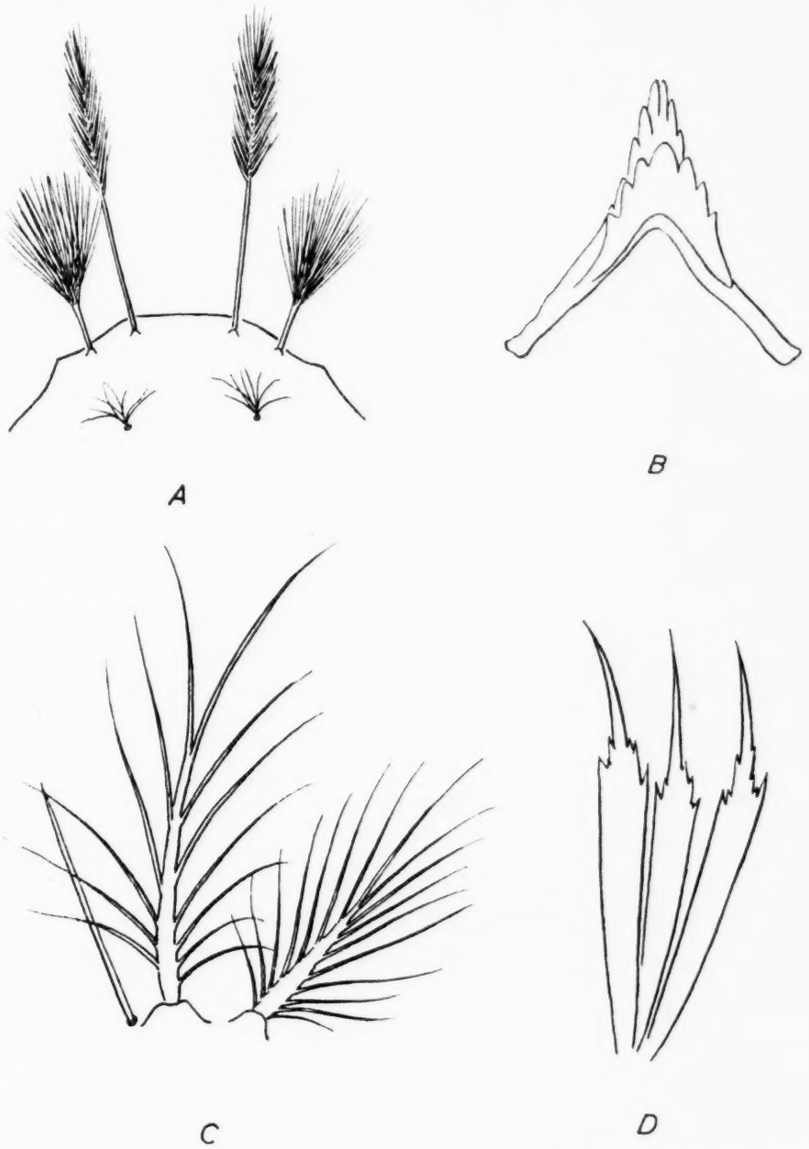


FIG. 16. *A. philippinensis* Ludlow. Larval details. *A.*—Clypeal hairs; *B.*—Mentum and submentum; *C.*—Shoulder hairs of prothorax; *D.*—Leaflets of palmate hair of segment V.

Spine. VIII, 10–11 branched, about 1/2 length of segment; VII, attenuated, 1/3 to 1/2 length of segment; VI and V, attenuated, progressively shorter; IV, short, blunt, 1/6 to 1/5 length of segment; III, about 1/2 length of preceding spine.

Hair B. V–VII, delicate, about 1/2 length of segment, 2- or 3-branched from near base ; III–IV, 5-branched.

Hair C. V-VII, simple, about the length of segment ; III-IV, 3–5 branched.

Hair No. 4. III–VII, delicate, about 1/3 length of segment, 2–4 branched.

LARVA. In life, dark greenish-grey to nearly black, with silvery-white spots. Average length of a full-grown larva 4·7 mm.

Head. Fig. 16, *A*, *B*. Inner clypeal hair 0·23 mm., its distal one-third to two-thirds conspicuously frayed ; its lateral branches 15–20 in number and about 1/6 to 1/5 of the length of main hair, outer clypeal 0·10 mm., broom-like, the main hair first split into 2 or 3, which again subdivide into 20-30 long branches ; sometimes the stem-hair does not subdivide twice but splits directly into these long branches. The distance between the inner clypeal is twice that between the inner and the outer clypeal. Posterior clypeal slightly external to inner clypeal, split into 7–8 branches, like a fan, in one plane. Innermost frontal hair long, reaching to about anterior end of head ; intermediate and outer frontal hair shorter, about half as long. Inner sutural 3–4 branched ; outer sutural 5–6 branched. Mentum with three teeth on either side of median tooth, third tooth smaller and placed further back than the others.

Thorax. The shoulder hairs of prothorax as shown in fig. 16, *C*.

Abdomen. Palmate hairs on I poorly developed, having only 3 or 4 lanceolate leaflets not differentiated into blade and filament. Palmate hairs on II–VII well developed, having 7–13 leaflets on II, 13–20 on III–VII. Serrations of leaflets well marked ; filament about 1/3 length of blade (fig. 16, *D*).

This species has not previously been recorded from China or from Formosa. Its occurrence in Hainan, however, appears to be quite common, judging by the many breeding-places found and the large number of larvae breeding in them. Adults were, however, not found in houses, although several searches were made in places near the breeding-ground.

BREEDING-PLACES. In Hainan *A. philippinensis* breeds especially in association with rice-fields. Out of the nine cultures from which this species was reared, six were from rice-fields, the remaining three coming from grassy ground flooded with water during the rainy season. Thirty male and 30 female adults were bred.

REFERENCES

BUDDLE, R. (1928). Entomological notes on the Canton delta. *Jl. Roy. Nav. Med. Serv.*, **14**, 190.

CHRISTOPHERS, S. R. (1916). A revision of the nomenclature of Indian Anophelini. *Ind. Jl. Med. Res.*, **3**, 454.

——— (1933). The fauna of British India. Diptera, vol. 4 : Family Culicidae, tribe Anophelini. London.

——— and KHAZAN CHAND (1916). A tree-hole breeding *Anopheles* from southern India : *A. (Coelodiazesis) culiciformis*, Cogill. *Ind. Jl. Med. Res.*, **3**, 638.

EDWARDS, F. W. (1915). The *Stegomyia* survey in Hong Kong. *Bull. Ent. Res.*, **6**, 67.

——— (1922). A revision of the mosquitoes of the Palaearctic region. *Ibid.*, **12**, 263.

EVANS, A. M. (1930). On certain distinguishing characters observed in *Anopheles funestus* Giles *Ann. Trop. Med. & Parasitol.*, **24**, 587.

FAUST, E. C. (1926). A preliminary check list of the mosquitoes of the Sino-Japanese areas. *China Med. Jl.*, **40**, 142.

FENG, L. C. (1932). Investigations on malaria and its transmission in Amoy, Fukian Province, South China. *Jl. Trop. Med. & Hyg.*, **35**, 198.

——— (1935). The present status of the knowledge of the mosquitoes of China and their relation to human diseases. *Chinese Med. Jl.*, **49**, 1183.

GASCHEN, H. (1934). Recherches entomologiques dans la province du Yunnan. *Bull. Soc. Méd.-Chir. Indochine*, **12**, 873.

GATER, B. A. R. (1935). Aids to the identification of anopheline imagines in Malaya. Singapore.

HO, CH'I (1938). A new tree-hole breeding *Anopheles* from the island of Hainan. *Ann. Trop. Med. & Parasitol.*, **32**, 279.

JACKSON, R. B. (1933). Annual report of the work of the Malaria Bureau for the year 1932. *Hong Kong Med. & San. Rep. for* 1932, App. B., p. 126.

——— (1934). Annual report of the work of the Malaria Bureau for the year 1933. *Ibid.*, 1933, App. B., p. 137.

——— (1935). The anophelines of the Colony of Hong Kong. *Trans. 9th Congr. Far East. Assoc. Trop. Med.*, **2**, 27.

KING, W. V. (1932). Three Philippine *Anopheles* of the *funestus-minimus* subgroup. *Philipp. Jl. Sci.*, **48**, 485.

LAMBORN, W. A. (1922). The mosquitos of some ports of China and Japan. *Bull. Ent. Res.*, **12**, 401.

LEE, C. U. (1932). A survey of the mosquitoes and their breeding habits in the Amoy region. *Ann. Rep. Mar. Biol. Assoc. China*, **1**, 75.

PURI, I. M. (1929). A new tree-hole breeding *Anopheles* from south India—*Anopheles sintoni* sp. nov.—and a revised description of the larva of *A. culiciformis* Cogill. *Ind. Jl. Med. Res.*, **17**, 397.

——— (1931). Larvae of anopheline mosquitoes, with full description of those of the Indian species. *Ind. Med. Res. Mem.*, **21**.

RILEY, W. A. (1932*a*). An annotated list of Canton, China, mosquitoes, based on collections by the late C. W. Howard. *Lingnan Sci. Jl.*, **11**, 25.

——— (1932*b*). Anopheline mosquitoes from the island of Hainan. *Ibid.*, **11**, 468.

——— and WU LIANG-YU (1932). The anopheline mosquitoes of China. *Ibid.*, **11**, 175.

SENEVET, G. (1931). Contribution à l'étude des nymphes d'anophélinés. *Arch. Inst. Pasteur Algérie*, **9**, 17.

SEVERN, A. G. M. (1926). The mosquitoes of Hongkong. *Caduceus*, **5**, 5.

TOUMANOFF, C. (1934). Observations sur les habitudes trophiques des anophélines de la colonie de Hong-Kong. *Bull. Soc. Path. Exot.*, **27**, 745.

WU LIANG-YU (1935). Investigations on natural infections of anopheline mosquitoes with malarial parasites in Canton. *Lingnan Sci. Jl.*, **14**, 691.

——— (1936). A study of anopheline larvae of Kwangtung Province, with notes on their relation to the incidence of malaria. *Ibid.*, **15**, 265.

YAMADA, S. (1924). A revision of the adult anopheline mosquitoes of Japan : systematic descriptions, their habits and their relations to human diseases, together with an account of three new species. *Sci. Rep. Inst. Infect. Dis. Tokyo Univ.*, **3**, 215.

——— (1925). A revision of the adult anopheline mosquitoes of Japan : systematic descriptions, their habits and their relations to human diseases. pt. II. *Ibid.*, **4**, 447.

THE SIZE OF APERTURE NECESSARY IN SCREENCLOTH INTENDED FOR THE PROTECTION OF DWELLINGS IN WEST AFRICA

BY

T. H. DAVEY

AND

R. M. GORDON

(*From the Sir Alfred Lewis Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication October 27th*, 1938)

INTRODUCTION

A considerable literature exists concerning the size of aperture and type of wire most suitable for screencloth intended for use in the tropics. In West Africa, however, the slow development of the colonies appears to have prevented proper attention being paid to this comparatively recent innovation. Blacklock (1935) published a paper entitled 'Screencloth for Houses in the Tropics'. In this interesting article he pointed out the future importance of screening in West Africa, reviewed briefly the cost of different types of metal gauze, and discussed the great advantages to be gained by adopting only two sizes of wire mesh, the smaller mesh being intended for screening water-containers and the larger mesh for houses. In connection with the latter, Blacklock pointed out that MacArthur (1923), as a result of experiments with *A.* (*S.*) *aegypti*, came to the conclusion that a wire screencloth having 14 meshes to the linear inch, and composed of wire of no. 30 Imperial Standard Wire Gauge, was entirely adequate. Blacklock, therefore, suggested the use of this mesh for screening houses in West Africa. His suggestion was supported by the Government Entomologist, Uganda, who in the Annual Medical and Sanitary Report for the year 1935 stated that he had carried out experiments with *A. funestus* and found that this species was unable to pass through wire gauze of the standard suggested by MacArthur. However, in July, 1937, the writers of the present article received a communication from the Gambian Government, stating that a consignment of wire gauze of the mesh just described, which had been imported for screening a new Government hospital, was suspected of allowing mosquitoes to penetrate. We were able to confirm this suspicion, and communicated our results to Professor Blacklock, who in 1937 published a further note, drawing attention to the fact that screencloth of 14 mesh and composed of no. 30 I.S.W.G. wire might not prove safe for houses in West Africa; and at the same time he arranged for experiments to be carried out with specimens of screencloth of different apertures, which he sent to India and elsewhere.

In the West African colonies, the chief transmitters of both malaria and filariasis are *A. gambiae* and *A. funestus*, and their infection-rate is extremely high. Thus, Gordon and others (1932) have shown that at a village in the vicinity of Freetown, Sierra Leone, 16·3 per cent. of *A. gambiae* are infected with malaria and 14·6 per cent. with *Microfilaria bancrofti*. *A. funestus* from the same districts show 7·0 per cent. infected with malaria and 8·0 per cent. with microfilaria, these figures referring respectively to salivary gland and/or oöcyst infections in the case of malaria, and to all forms of infections in the case of filariasis.

With so high an infection-rate amongst such common mosquitoes, it is obviously of the utmost importance to use screencloth which is proof against penetration by either species. In this connection, it is interesting to note that Mulligan and Majid (1932) advised the use in India of a screencloth which, when tested in a similar manner to that which we are about to describe, allowed 5 out of a total of 176 *A. culicifacies* to escape through this mesh during an observation-period of 72 hours. They recommend the use of this screencloth, in spite of the fact that in the same paper they refer to *A. culicifacies* as ' one of the most dangerous malaria carriers in India '. In their opinion, the advantages to be gained from the greater amount of air and light admitted by this screen outweigh the risk incurred by using so comparatively wide a mesh. However true this may be of India, we do not consider that it applies in West Africa, where the high infection-rate and the great density of the anopheline population render it essential to use only completely safe screening.

SCOPE AND TECHNIQUE

A total of some 16,000 mosquitoes was tested against screencloths having apertures of 0·043, 0·047, 0·050 and 0·059 of an inch, the mosquitoes chiefly used being *A. funestus* and *A. gambiae*.

A. funestus is, of course, a much smaller species than *A. gambiae* (it is probably the smallest of the anophelines transmitting malaria and filariasis in West Africa); it was therefore more commonly used in our experiments than any other species. We carried out no experiments with *Aëdes* (*S.*) *aegypti*, since the experiments which had already been published by other authors appeared to us at the time to be adequate. We did, however, carry out a limited number of experiments with other small species of *Aëdes*, such as *A.* (*S.*) *vittatus* and *A.* (*S.*) *simpsoni*, which may be potential vectors of yellow fever.

In order to imitate natural conditions as far as possible, we made use of mosquitoes captured in houses, and used bred mosquitoes only when wild ones were unobtainable. All experiments with *A. gambiae* and *A. funestus* were conducted with wild mosquitoes; when bred mosquitoes were used, they were kept for a minimum period of 24 hours before being tested, in order to allow for hardening of the exoskeleton.

The apparatus illustrated in fig. 1 was used in all our experiments, and we believe that the accompanying diagram is sufficiently clear not to need elaboration in the text. The section of cage containing the mosquitoes was covered with a cloth, and the end containing the animal, together with the section containing

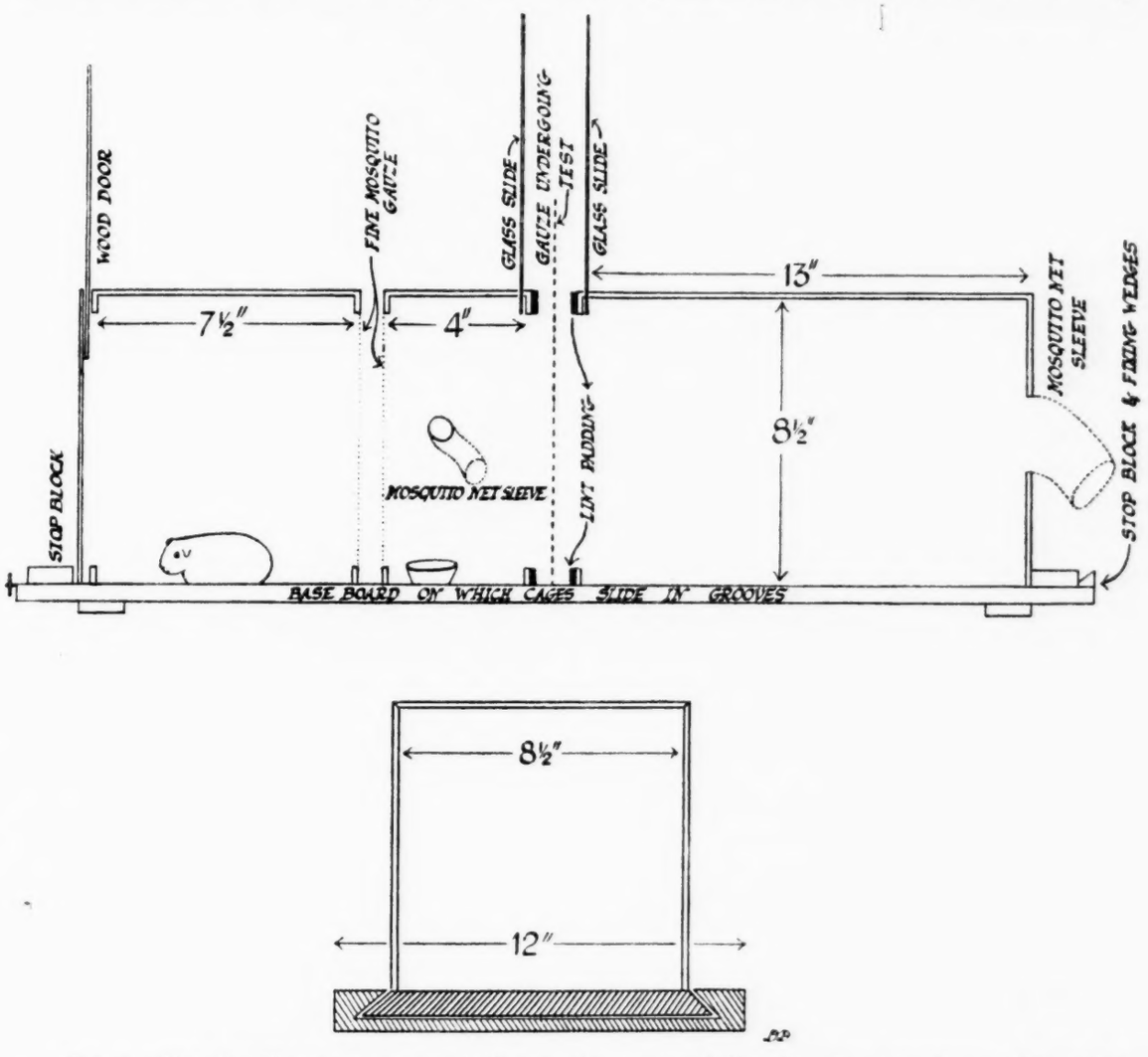


FIG. 1. Showing the apparatus used when testing screencloths of various apertures. It consists of three separate wooden cages protected by very small mesh screencloth. The cages are shown slightly separated in the drawing, but they can be joined together on sliding them along the grooves in the baseboard. When this is done, the screencloth undergoing the test is clamped between the right-hand cage and the small central compartment. A guinea-pig is placed in the left-hand cage, and sugar and water in the central compartment. The glass slides are withdrawn at the commencement of the experiment and replaced at the completion of the experiment, when counts are made of the mosquitoes in the compartments.

the sugar and water, was directed towards the light—the window during the day, and an electric lamp at night. This was done in order to provide a light tropism, as well as the food and water tropisms.

The number of mosquitoes which passed through the wire gauze of the mesh used in our experiments was so small that we were never given the

opportunity of actually seeing the feat accomplished. We therefore devised the simple apparatus shown in fig. 2, and employed 12-mesh wire gauze of 30 S.W.G. Through this mesh mosquitoes escaped with fair frequency, and the narrowness of the glass cage allowed their movements to be studied with accuracy under a magnifying-glass.

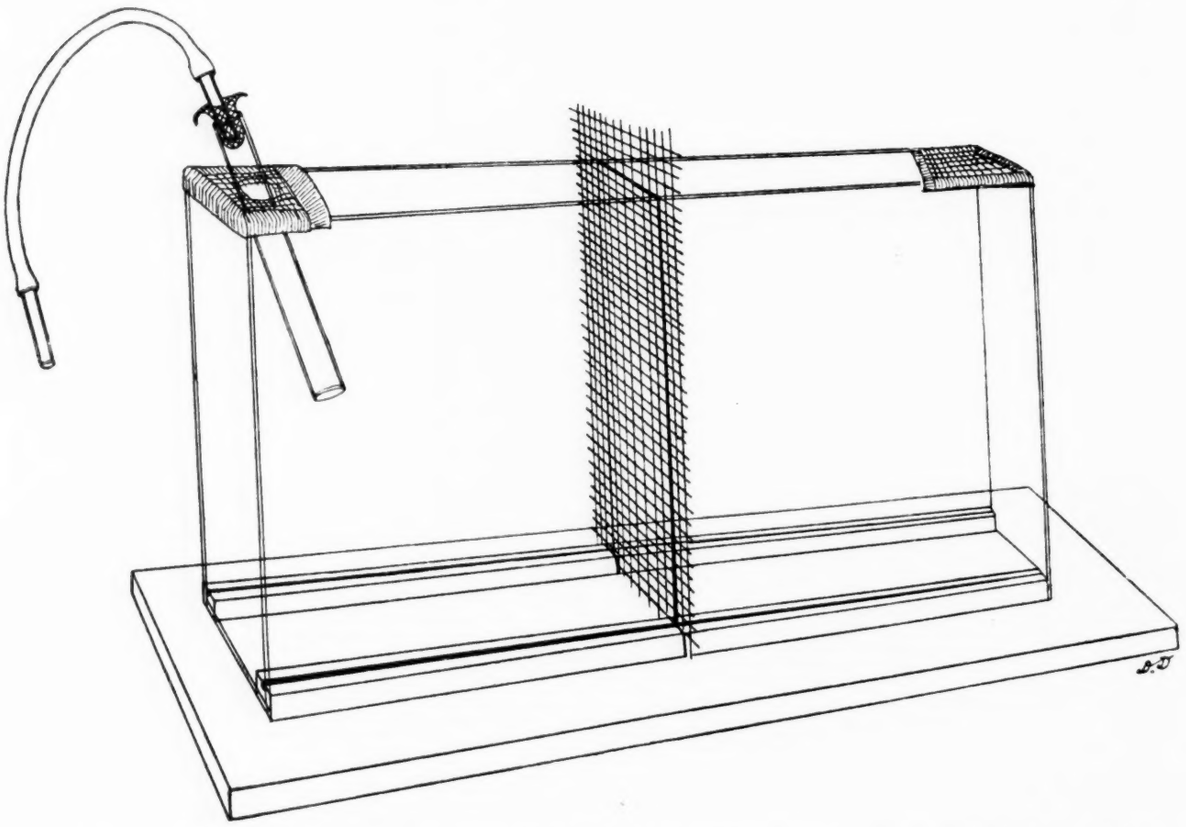


FIG. 2. Showing the apparatus used to observe the movements of mosquitoes when passing through screencloth ; the two cells shown are made of glass separated by a strip of metal screencloth. By breathing through the tube shown on the left of the drawing, the mosquitoes are stimulated to pass through the wire mesh to the forward compartment, and their movements while so doing are observed under a magnifying-glass.

RESULTS OBTAINED

Experiments 1–12. These were carried out with 14 × 14 × 30 S.W.G. screencloth having apertures of 0·059 of an inch. In these experiments the mosquitoes were identified and counted before being placed in the testing-cage. As a result, the protocols, although strictly comparable with the other experiments, are presented in a slightly different manner.

It can be seen from Table I that, out of a total of 160 *A. gambiae*, 2 (1·3 per cent.) penetrated, while of 801 *A. funestus* 11 (1·4 per cent.) passed through an aperture of 0·059 inches. Amongst 573 *Aëdes* (*S.*) *vittatus* tested, 12 (2·1 per cent.) escaped. Only 18 *Aëdes* (*S.*) *simpsoni* were used, but the number

TABLE I

Showing the results of testing 1,579 mosquitoes, of various species, against wire gauze having apertures with a diameter of 0·059 of an inch (woven wire cloth 14 × 14 × 30 S.W.G.)

Species	Recovered dead		Recovered alive		Passed through		Not accounted for		Total
	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	
A. gambiae	10	73	1	53	—	2	4	17	160
A. funestus	159	393	16	198	—	11	10	14	801
A. rufipes	0	1	—	1	—	—	—	3	5
A. rhodesiensis	1	2	—	—	—	—	—	—	3
Aëdes (S.) simpsoni	4	4	—	2	3	1	2	2	18
Aëdes (S.) vittatus	195	204	66	94	3	9	2	—	573
C. decens	6	5	—	—	—	—	1	1	13
C. cinereus	—	2	—	—	—	—	1	—	3
C. duttoni	—	1	—	—	—	—	—	—	1
C. nebulosus	—	2	—	—	—	—	—	—	2
	375	687	83	348	6	23	20	37	1,579

which passed through (4, i.e., 22·2 per cent.) was so large a proportion as to prove that the size of mesh did not give adequate protection. None of the remaining species passed through an aperture of 0·059 of an inch, but the numbers tested are inadequate to allow of any positive conclusion being arrived at.

Experiments 13–18. These were carried out with 16 × 16 × 30 S.W.G. screencloth having apertures of 0·050 of an inch.

A total of 2,935 unidentified mosquitoes were put into the testing-cage, and the dead were removed and identified on subsequent days, with the following results.

Out of a total of 2,935 mosquitoes put into the cage (as shown in Table II), 11 *A. funestus* females passed through the gauze, i.e., 0·38 per cent. of the total mosquitoes or 0·4 per cent. of the *A. funestus*. No other species passed through the gauze, but it should be noted that *A. funestus* was the smallest species tested, and also formed 96 per cent. of the total tested. Of the total mosquitoes tested 41 were not recovered. These probably either died in the cage and were ground to powder in the slots, or were removed by ants which on occasions were introduced by error with the guinea-pig's food. Details of the species tested are given in Table II.

Experiments 19–24. Carried out with 16 × 16 × 28 S.W.G. screencloth having apertures of 0·047 of an inch.

A total of 7,591 unidentified mosquitoes were put into the testing-cage, and the experiments were carried out as on previous occasions.

TABLE II

Showing the results of testing 2,935 mosquitoes against wire gauze having apertures with a diameter of 0·050 of an inch (woven wire cloth 16 × 16 × 30 S.W.G.)

Species	Recovered dead			Recovered alive			Passed through	Total
	♂♂	♀♀	Total	♂♂	♀♀	Total		
A. gambiae	17	41	58	1	44	45	—	103
A. funestus	302	2,118	2,420	10	329	339	11	2,770
A. rufipes	—	3	3	—	—	—	—	3
C. decens	—	7 { 4 / 3 }	7 { 4 / 3 }	—	—	—	—	4
C. decens var. *invidiosus* ...	—			—	1	1	—	4
C. nebulosus	1	4	5	—	2	2	—	7
C. cinereus...	—	1	1	1	1	2	—	3
	320	2,174	2,494	12	377	389	11	2,894

TABLE III

Showing the results of testing 7,591 mosquitoes against wire gauze having apertures with a diameter of 0·047 of an inch (woven wire cloth 16 × 16 × 28 S.W.G.)

Species	Recovered dead		Passed through	Total
	♂♂	♀♀		
A. costalis	142	2,321	—	2,463
A. funestus	523	4,351	—	4,874
C. decens	—	2	—	2
	665	6,674	—	7,339

Table III shows that, of a total of 7,591 mosquitoes added to the cage, 7,339 mosquitoes were recovered and identified as shown above. Two hundred and fifty-two were not recovered, mainly owing to their being destroyed by ants introduced into the cage with the guinea-pig's food. None of the mosquitoes succeeded in passing through the gauze.

Experiments 25–30. Carried out with 18 × 18 × 30 S.W.G. screencloth having apertures of 0·043 of an inch.

A total of 4,301 unidentified mosquitoes were put into the testing-cage and dealt with as in previous experiments. Of this total, 4,188 were recovered and 113 were not accounted for. None of the mosquitoes passed through the gauze. Details of the species tested are given in the table below.

TABLE IV

Showing the results of testing 4,301 mosquitoes against wire gauze having apertures with a diameter of 0·043 of an inch (woven wire cloth 18 × 18 × 30 S.W.G.)

Species	Recovered dead			Recovered alive			Passed through	Total
	♂♂	♀♀	Total	♂♂	♀♀	Total		
A. funestus	737	3,358	4,095	1	37	38	—	4,133
A. costalis	40	5	45	—	—	—	—	45
C. decens var. *invidiosus* ...	1	7	8	—	—	—	—	8
C. cinereus	1	1	2	—	—	—	—	2
	779	3,371	4,150	1	37	38	—	4,188

PROCESS BY WHICH THE MOSQUITO PASSES THROUGH A NARROW APERTURE

It is of some interest to consider the actual process by which the mosquito succeeds in working its way through the narrow apertures of the wire gauze. Using the apparatus which we have already described and illustrated in fig. 2, we were enabled to watch with a magnifying-glass a series of escapes. The technique was curiously uniform, and was repeated with only very slight variations in every instance.

The mosquito first of all alights on the wire gauze and probes the aperture with its head and proboscis, later pressing the fore part of the thorax through the opening. It then completely withdraws the head and proboscis from the opening, and deliberately passes through one, or sometimes both, forelegs. It then reintroduces the head and thorax, and at this stage it always rotates the thorax, so that its greatest diameter lies in the diagonal of the square formed by the wires. The movements which now follow, and allow of the passage of the thorax, are somewhat difficult to make out, and consist of a wriggling movement, apparently assisted by pulling with the released foreleg or legs. Once the thorax is through, the remaining four or five legs may be seen closely pressed against the abdomen, and while in this position the insect appears generally to fall through the aperture. Indeed, we have sometimes noticed them drop several inches before expanding their wings. The stages are diagrammatically shown

in fig. 3. We carried out these observations because it has been suggested (Uganda Annual Report of the Medical Department for 1935) that it is the length of the mosquito's legs and not the size of the body which determines its ability to pass through narrow apertures.

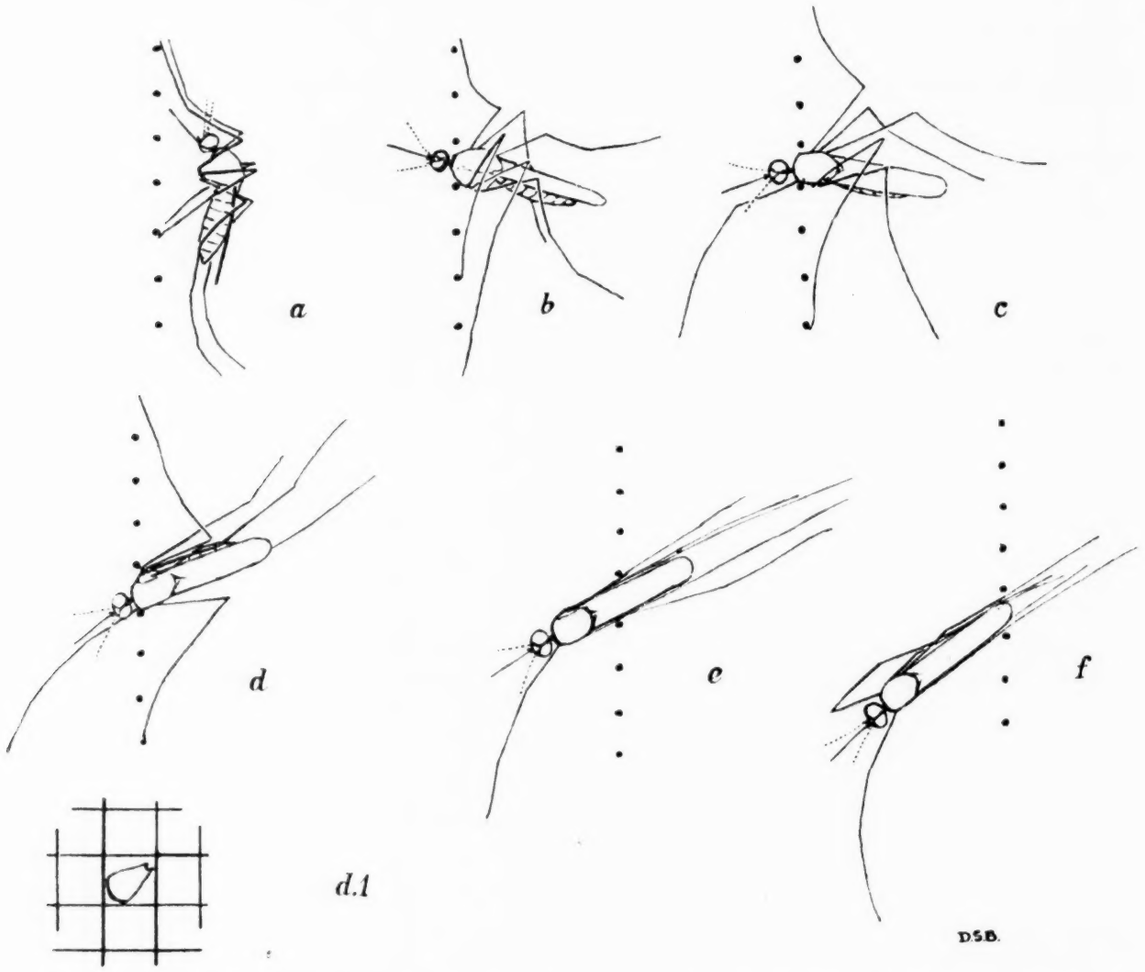


FIG. 3. Showing the various stages by which a mosquito passes through a narrow aperture in a screencloth ; *d, i,* represents, diagrammatically, the turning of the thorax, so that its long axis forms a diagonal in the square opening of the gauze.

DISCUSSION

The more important experimental work previously published on this subject may briefly be summarized as follows :

Le Prince and Orenstein (1916), working in Panama, reached the conclusion that 16-mesh gauze of 28 B.W.G. will exclude anophelines and will only permit the passage of *A.* (*S.*) *aegypti* ' under the stress of circumstances ', while 17-mesh gauze of no. 30 B.W.G. will exclude *A.* (*S.*) *aegypti.* They do not furnish details of their experiments. MacArthur (1923) experimented with

A. (*S.*) *aegypti* bred in England ; numerous experiments were carried out and resulted in a single female passing through 12-mesh wire gauze of 28 I.S.W.G. No mosquitoes passed through the 14-mesh wire gauze of 30 I.S.W.G. In MacArthur's experiments, the attraction to escape was provided either by a hand held near the screen or by a guinea-pig, sugar and water being also present. Gage (1925) carried out a series of experiments in Carolina with *A. punctipennis*, *A. quadrimaculatus* and *A. crucians*. In his experiments the anophelines were offered a choice of escape through three equal-sized apertures guarded by, respectively, 12-mesh 30 S.W.G., 14-mesh 30 S.W.G. and 16-mesh 31 S.W.G. No mosquitoes escaped through 16- or 14-mesh, but four *A. crucians* passed through the 12-mesh. We do not consider that this proves that the species of anophelines experimented with were unable to pass through the smaller apertures, since it appears reasonable to think that, when offered the choice of three apertures, they would select the largest. Earle (1930), when dealing with the control of malaria in Porto Rico, reached the conclusion that ' 16-mesh cloth ·009- or ·010-wire [i.e., with 33 or 34 S.W.G.] is sufficient to keep out practically all mosquitoes '. Mulligan and Majid (1932) have carried out a series of very carefully controlled experiments in India, using *A. culicifacies* as the test mosquito, and they found that complete protection was only provided by a wire gauze of 16 mesh and 26 S.W.G. The Government Entomologist, Uganda, in his annual report for 1935, states that hungry female *A. funestus* in every instance failed to pass through wire gauze of 14 × 14 mesh, 30 S.W.G.

Covell (1931) and Mulligan and Majid (1932) have drawn attention to the confusion caused by the use of various formulae for designating particular types of screencloth. They rightly emphasize the fact that, in experiments undertaken to determine what mesh will prevent the passage of mosquitoes, the important point is the actual size of the aperture. By using this method we can reduce our own figures and those of all previous observers, who have supplied sufficient data, to a common term, i.e., the diameter of the aperture, expressed in fractions of an inch. This has been done in Table V.

It will be seen from Table V that there are no published records of mosquitoes having passed through screencloths with apertures of less than 0·048 of an inch in diameter. The fact that a mosquito has passed through an aperture of a certain diameter indicates, of course, that the screencloth is not proof against the species of mosquito used in that experiment. A negative result, however, must be accepted with some caution, unless a large number of mosquitoes have been employed in the experiment. In certain districts in West Africa it is probable that several hundred mosquitoes enter each native house during the course of a single night. If such buildings were screened with gauze which allowed passage to even so small a proportion as 0·5 per cent., the total number of mosquitoes entering the dwelling over a long period would be considerable.

TABLE V

Showing the results of experiments carried out by various observers to ascertain what proportion of mosquitoes of different species will pass through screencloths of various apertures

Diameter of aperture, expressed in inches	Species of mosquito tested	No. tested	Authority and date	No. and percentage which escaped
0·070	*A. culicifacies*	230	Mulligan & Majid (1932)	40 (17·3%)
	A. crucians	251	Gage (1925)	4 (1·6%)
	A. punctipennis	114	,, ,,	Nil
	A. quadrimaculatus	148	,, ,,	Nil
0·065	*A. culicifacies*	224	Mulligan & Majid (1932)	16 (7·1%)
0·060	*A. culicifacies*	229	Mulligan & Majid (1932)	20 (8·7%)
0·059	*A. punctipennis*	114	Gage (1925)	Nil
	A. quadrimaculatus	48	,, ,,	Nil
	A. crucians	251	,, ,,	Nil
	Aëdes (S.) aegypti	?	MacArthur (1923)	Nil
	Aëdes (S.) vittatus	573	Davey & Gordon (1938)	12 (2·1%)
	Aëdes (S.) simpsoni	18	,, ,, ,,	4 (22·2%)
	A. gambiae	160	,, ,, ,,	2 (1·25%)
	A. funestus	801	,, ,, ,,	11 (1·4%)
	A. funestus	?	Gov't. Entomologist Uganda (1936)	Nil
0·055	*A. culicifacies*	176	Mulligan & Majid (1932)	5 (2·8%)
0·053	?	?	Earle (1930)	Keeps out 'practically all mosquitoes'
0·050	*A. punctipennis*	114	Gage (1925)	Nil
	A. quadrimaculatus	48	,, ,,	Nil
	A. crucians	251	,, ,,	Nil
	A. culicifacies	217	Mulligan & Majid (1932)	3 (1·4%)
	A. gambiae	103	Davey & Gordon (1938)	Nil
	A. funestus	2,770	,, ,, ,,	11 (0·4%)
0·048	*A. (S.) aegypti*	?	Le Prince & Orenstein (1916)	It 'will permit the passage of *Aëdes calopus* only under stress of circumstances'
	Anopheles	?	,, ,, ,,	Nil
0·047	*A. gambiae*	2,463	Davey & Gordon (1938)	Nil
	A. funestus	4,874	,, ,, ,,	Nil
0·046	*A. (S.) aegypti*	?	Le Prince & Orenstein (1916)	'Will exclude this species'
0·045	*A. culicifacies*	319	Mulligan & Majid (1932)	Nil
0·043	*A. gambiae*	45	Davey & Gordon (1938)	Nil
	A. funestus	4,133	,, ,, ,,	Nil
0·035	*A. culicifacies*	220	Mulligan & Majid (1932)	Nil

Table V shows that, amongst the Anophelini, *A. culicifacies* in India and *A. gambiae* and *A. funestus* in West Africa appear to be excluded by screencloth having apertures of a diameter of 0·047 of an inch or less. Gage's experiments with *A. punctipennis*, *A. crucians* and *A. quadrimaculatus* appear to show that these species are excluded by a larger diameter of mesh, but for reasons already stated the present writers believe that this conclusion cannot be regarded as proved. As regards the tribe Culicini, the number of experiments undertaken, and the number and species of mosquitoes used in the experiments, are not sufficient to warrant any statement being made as to the size of aperture necessary to prevent penetration. It is true that in the case of the Aëdes group, MacArthur's (1923) observations with *Aëdes* (*S.*) *aegypti* appear to show that an aperture of 0·059 of an inch is sufficient to exclude this species, but the earlier work of Le Prince and Orenstein (1916) suggests that only a much finer mesh can be regarded as safe. Our own experiments prove that a high proportion of *Aëdes* (*S.*) *vittatus* and *Aëdes* (*S.*) *simpsoni* can pass through the 0·059 aperture which MacArthur has found to be proof against *Aëdes* (*S.*) *aegypti*.

SUMMARY AND CONCLUSIONS

Experiments were carried out in West Africa with some 16,000 local mosquitoes, to test their ability to pass through screencloths of various apertures. *A. gambiae* and *A. funestus* were found to be capable of passing through a screencloth with apertures 0·059 of an inch in diameter, while some *A. funestus* escaped through apertures as small as 0·050 of an inch in diameter. The result of these experiments appears to show that the maximum size of aperture advisable for screening houses against anophelines in West Africa must not exceed 0·047 of an inch, which is represented by a standard screencloth of mesh 16 × 16, composed of wire of 28 S.W.G. Experiments with the Aëdes group proved that *Aëdes* (*S.*) *vittatus* and *Aëdes* (*S.*) *simpsoni* can penetrate through wire-gauze apertures of 0·059 of an inch. No experiments were carried out to ascertain if they can pass through screencloth of a smaller mesh.

Observations were made on the process by which mosquitoes squeeze their way through narrow apertures. The results suggest that their inability to pass through any particular size of aperture is directly dependent on the dorso-ventral diameter of the thorax.

ACKNOWLEDGEMENTS.—The writers are indebted to Messrs. N. Greening and Sons Ltd., Britannia Works, Warrington, for supplying samples of the various standard screencloths which were tested in the experiments. They are also indebted to Mr. D. S. Bertram, B.Sc., and to Mr. David Dagnall, both of the Liverpool School of Tropical Medicine, for the drawings accompanying this paper.

REFERENCES

BLACKLOCK, D. B. (1935). Screencloth for houses in the tropics. *Ann. Trop. Med. & Parasitol.*, **29**, 261.

——— (1937). Screencloth for houses in the tropics. *Ibid.*, **31**, 447.

COVELL, G. (1931). Malaria control by anti-mosquito measures. Calcutta.

EARLE, W. C. (1930). Malaria in Porto Rico. *Amer. Jl. Trop. Med.*, **10**, 207.

GAGE, E. H. (1925). Observations on the passage of anopheline mosquitoes through screens of various-sized mesh. *Publ. Health Bull., Washington*, no. 156, p. 44.

GORDON, R. M., HICKS, E. P., DAVEY, T. H., and WATSON, M. (1932). A study of the house-haunting Culicidae occurring in Freetown, Sierra Leone; and of the part played by them in the transmission of certain tropical diseases, together with observations on the relationship of anophelines to housing, and the effects of anti-larval measures in Freetown. *Ann. Trop. Med. & Parasitol.*, **26**, 273.

LE PRINCE, J. A., and ORENSTEIN, A. J. (1916). Mosquito control in Panama. New York.

MACARTHUR, W. P. (1923). Mosquito netting. *Jl. Roy. Army Med. Corps*, **40**, 1.

MULLIGAN, H. W., and MAJID, J. A. (1932). Some experiments to determine the efficacy of wire gauze screens of different apertures in excluding anopheline mosquitoes in India. *Rec. Malaria Surv. Ind.*, **3**, 163.

UGANDA PROTECTORATE (1936). Annual report of the Government Entomologist for 1935. *Ann. Rep. Med. Dept. for* 1935, App. III, p. 69.

OBSERVATIONS ON THE BEHAVIOUR IN LABORATORY ANIMALS OF *TRYPANOSOMA CONGOLENSE* BRODEN, 1904

BY

H. R. BINNS, M.A. (CANTAB.), M.R.C.V.S.

(*Veterinary Department, Nyasaland*)

(*Received for publication November 2nd*, 1938)

The variation in the pathogenicity for laboratory animals of the trypanosome now generally known as *T. congolense* has frequently been recorded since the trypanosome was first described. It is not proposed in this note to quote the numerous observations on the behaviour of *T. congolense* in laboratory animals recorded by individual workers. It is sufficient to state that, among others, Laveran (1905), Bruce, Hamerton, Bateman, Mackie and Lady Bruce (1911*a* and 1911*b*), Blacklock and Yorke (1913), Delanoë (1914), and Bruce, Harvey, Hamerton and Lady Bruce (1914) all record the results of experimental inoculations of laboratory animals with the small aflagellar pathogenic trypanosome variously named *T. dimorphon*, *T. congolense*, *T. pecorum* and *T. nanum*. These observations indicate that there appears to be much variation in the pathogenicity of different strains of *T. congolense* for laboratory animals, and that as a rule passage of a particular strain through small animals tends to increase this pathogenicity. It is also shown that susceptibility to any particular strain varies both with the species and the individual. This variation in susceptibility and pathogenicity is manifested by the widely different results obtained when *T. congolense* is inoculated into laboratory animals, particularly when, as often happened in the case of workers in tropical Africa, the number of animals inoculated was small. As was clearly shown by Blacklock and Yorke (1913), these greatly varying results emphasize the fallibility of conclusions regarding the pathogenicity of *T. congolense* drawn from a small number of inoculations, and the danger of differentiating strains or species of trypanosomes on the results of such pathogenicity tests alone.

It is the purpose of this note to record the results of the inoculation into small laboratory animals, mainly white rats, of strains of *T. congolense* causing disease of cattle in the southern province of Nyasaland. The object of these inoculations was to obtain in laboratory animals strains of *T. congolense* for experimental investigation. Since, however, the inoculations give some indication of the behaviour in laboratory animals of seven strains of *T. congolense*, it is considered that the results of these inoculations are of interest.

TECHNIQUE. The procedure adopted was the same in all inoculations. Fresh blood-smears from an ox known to be infected were examined, and laboratory animals were inoculated only if active trypanosomes were found in

the circulation immediately before inoculation. About 10 c.cm. of blood were withdrawn from the jugular vein into an equal part of citrated saline solution, contained in a measuring-jar standing in water at blood-temperature. The amount of citrated blood inoculated varied from 1 to 2 c.cm. It was early noted that, in contradistinction to the observations of some workers, intraperitoneal inoculation did not appear to be more successful than subcutaneous inoculation, and most inoculations, both in the isolation and passage of trypanosomes, were made by the latter route. When all the laboratory animals had been inoculated

TABLE I

Showing the results of the inoculation of seven strains of *T. congolense* into laboratory animals

Strain	Date of inoculation	Animals inoculated	Animals infected	Incubation period in days	*Duration of infection from inoculation to death
Limbe	31.3.36	6 R	3 R	12 12 18	66 55 81
		2 G	2 G	12 18	43 33
		2 Rb	—	—	—
Namalanga	14.5.36	5 R	—	—	—
Midima	17.6.36	6 R	—	—	—
		2 G	—	—	—
		2 Rb	—	—	—
Port Herald ...	24.7.36	6 R	3 R	9 15 15	37 54 46
Chikwawa A ...	11.2.37	8 R	2 R	9 21	62 77
		2 G	1 G	18	39
Chikwawa B ...	4.8.37	6 R	—	—	—
Lisungwe	12.8.37	6 R	4 R	6 9 9 12	21 31 33 27
Chiromo	16.9.37	6 R	—	—	—
Lunzu A	14.4.38	12 R	3 R	12 15 18	38 33 49
		2 G	1 G	18	51
Lunzu B	21.6.38	12 R	8 R	9 12 12	44 39 31
				12 12 15	55 48 33
				15 21	57 42

R = rat; G = guinea-pig; Rb = rabbit.

*Recorded in the same order as the incubation periods in the previous column.

with bovine blood, a check examination of fresh smears of the inoculum was made for actively motile trypanosomes. Stained blood-films were prepared from the infected ox, and the presence of *T. congolense* was confirmed. Subsequent to inoculation, fresh smears of the blood of the animals were examined every third day for the presence of trypanosomes. It was not possible to make daily examinations, and consequently the incubation periods recorded in Tables I and II are only correct to within three days. The period during which inoculated

animals were observed before being discarded as not being infected was not recorded, but in every case smear examinations were continued for several weeks, and it is improbable that any infections were missed.

The results of these inoculations are summarized in Table I. The Table shows the species and number of laboratory animals which were inoculated with each strain, the number which became infected, the days elapsing between inoculation and the first detection of trypanosomes in the circulation, and the duration of the infections from inoculation to death. The names of the strains are those of the areas in which the outbreaks of bovine trypanosomiasis occurred.

TABLE II

Showing the results of the first passage of four of the strains referred to in Table I

Strain	Rats infected	Incubation period	Duration of infection	Remarks
Limbe... ...	3	9 15 15	39 36 46	The average duration of the 2nd to 5th passages was 28 days ; of the 6th to 11th passages was 21 days ; of the 12th to 16th passages was 16 days
Port Herald ...	2	9 12	23 28	The duration of the 2nd, 3rd and 4th passages averaged 19 days ; the 5th to 9th passages averaged 12 days. The strain was not maintained beyond the 9th passage
Chikwawa A ...	3	9 9 12	32 38 41	Average course of 2nd to 6th passages was 26 days. Since the 6th passage it has varied from 13 to 24 days
Lisungwe ...	4	6 6 6 9	11 11 11 11	All were found dead on 15.9.37, and the strain was lost

The following facts are of importance in the interpretation of the results set forth in Table I. The strains Limbe and Namalanga are almost certainly the same strain, the Limbe outbreak having resulted from the movement of infected cattle from Namalanga, with subsequent direct transmission by biting flies. Strains Chikwawa A and B are also, in all probability, the same strain. An outbreak of *T. congolense* disease was diagnosed at Chikwawa in February, 1937. A course of treatment was administered to the affected herd, resulting in the disappearance of symptoms and improvement in the condition of the herd. During July, 1937, there was a recurrence of disease in the same herd, the circumstances of which suggested a relapse of the previous outbreak rather than a fresh infection. The strains Lunzu A and B are very probably the same

strain, having been obtained from cattle in two adjacent herds, successively infected during one outbreak of trypanosomiasis. Larger numbers of rats were inoculated with the Lunzu than with the other strains, in order to attempt to obtain a more accurate indication of the pathogenicity of the strains, by reducing variation in the results due to individual susceptibility.

In Table II are summarized the results of the first passage in rats of four of the strains of *T. congolense* referred to in Table I. Four rats were inoculated with each strain. In the second and subsequent passages of the Limbe, Port Herald and Chikwawa A strains all laboratory animals inoculated became infected. Details of these passages are beyond the scope of this paper. It was observed that the virulence of all three strains was increased with passage, the incubation period and duration of infection being shortened ; an indication of this increase in virulence is given under ' Remarks ' in Table II. This increased pathogenicity for a particular species of laboratory animal, resulting from repeated passage through that animal, appeared to be diminished by passing the strain through a different species and then reintroducing it into the original animal.

DISCUSSION

The observations summarized in Tables I and II confirm the view that greatly varying results are obtained when natural strains of *T. congolense* are first inoculated into laboratory animals. Examination of the tables shows that there was a considerable difference in the susceptibility of the individual animals to these strains. Although this difference tends to mask the variation in pathogenicity of different strains of *T. congolense*, the observations recorded in this paper give some indication of this variation. The results set forth in Table II confirm the general observation that passage of trypanosomes through laboratory animals increases their pathogenicity for these animals.

Considering first the question of individual susceptibility, as shown by the Limbe, Port Herald, Chikwawa A, Lisungwe and Lunzu strains, the inoculation of a particular strain into a number of laboratory animals usually produces infection of a proportion only of these animals, suggesting that the resistance of the remainder was sufficiently great to prevent infection by a trypanosome of the pathogenicity exhibited by that strain. This point is well shown by the results obtained with the Lunzu A and B strains : inoculation on one occasion of twelve rats and two guinea-pigs produced infection of only three rats and one guinea-pig, whereas, of twelve rats inoculated two months later, eight became infected.

Inoculation of two rabbits, two guinea-pigs and six rats with the Midima strain, and six rats with the Chiromo strain, did not produce infection in any of these animals. These results might be interpreted as indicating that these two strains are not pathogenic for these animals, and indeed such an interpretation has been put upon similar results in the past. However, in view of the small

number of animals inoculated, it is suggested that it is not possible to state whether the failure to produce infection was due to non-pathogenicity of these strains or to the fact that all the animals inoculated happened to be sufficiently resistant to prevent infection. This opinion is supported by the results of the inoculation of the Limbe and Namalanga strains on the one hand, and of the Chikwawa A and B strains on the other. Of five rats inoculated with the Namalanga strain, and six rats inoculated with Chikwawa B strain, none became infected, and it might be assumed that these strains were therefore non-pathogenic for rats. But the results of inoculation of the Chikwawa A and Limbe strains, which were in all probability the same as the Chikwawa B and Namalanga strains, show that these strains were pathogenic for rats and guinea-pigs. It is considered that these observations demonstrate clearly the danger of basing conclusions regarding the pathogenicity of trypanosomes on a limited number of experimental inoculations. Individual susceptibility is also manifested by the variations in incubation period and duration of disease of different animals inoculated with the same strain.

In view of the difference in susceptibility illustrated in the preceding paragraphs, the degree of pathogenicity of any particular strain is not readily determined. The proportion of the total number of inoculations which produce infection is obviously not a reliable criterion of pathogenicity. However, the incubation period and the duration of infection do give evidence of pathogenicity, and the results here reported give some indication of the variation in pathogenicity of different strains. Examination of Tables I and II shows that the Lisungwe strain appears to be highly pathogenic for rats. The four rats which became infected all showed brief incubation periods, and the average duration of infection was 28 days. In the first passage of the Lisungwe strain, the incubation periods were again short, and all four rats died eleven days after inoculation, which in the experience of the writer represents a very rapid course in a first passage. The Limbe strain seems to be of low pathogenicity, the incubation periods and courses of infection, both in the isolation and passage of the strain, being of comparatively long duration. The results of the inoculation and passage of the Chikwawa A and Lunzu strains suggested that these were of moderate pathogenicity.

SUMMARY

The results of the inoculation into laboratory animals of seven strains of *T. congolense*, causing disease of cattle in Nyasaland, are recorded.

These results show that there is considerable difference in the susceptibility of individual animals to this trypanosome, and indicate that different strains of *T. congolense* appear to vary in pathogenicity.

It is considered that the observations recorded in this communication give striking evidence of the fallibility of conclusions regarding the pathogenicity of trypanosomes based on the results of a small number of inoculations.

REFERENCES

BLACKLOCK, B., and YORKE, W. (1913). The probable identity of *Trypanosoma congolense* (Broden) and *T. nanum* (Laveran). *Ann. Trop. Med. & Parasitol.*, **7**, 603.

BRUCE, SIR D., HAMERTON, A. E., BATEMAN, H. R., MACKIE, F. P., and LADY BRUCE (1911*a*). Trypanosome diseases of domestic animals in Uganda : *Trypanosoma pecorum*, sp.nov. *Rep. Sleeping Sickness Comm. Roy. Soc.*, XI, 128.

——— ——— ——— ——— ——— (1911*b*). Trypanosome diseases of domestic animals in Uganda : *Trypanosoma nanum* (Laveran). *Ibid.*, XI, 164.

——— HARVEY, D., HAMERTON, A. E., and LADY BRUCE (1914). Trypanosome diseases of domestic animals in Nyasaland. III : *Trypanosoma pecorum*. *Ibid.*, XV, 78.

DELANOË, P. (1914). Des variations du pouvoir infectieux et de la virulence du *Trypan. dimorphon*, à partir d'infections naturelles présentées par les boeufs et les moutons. *Bull. Soc. Path. Exot.*, **7**, 58. (Reviewed in *Trop. Dis. Bull.*, **3**, 253.)

LAVERAN, A. (1905). Note pour servir à l'histoire des trypanosomiases du Soudan anglo-égyptien. *C. R. Soc. Biol.*, **58**, 292. (Quoted from Wenyon, 1926.)

WENYON, C. M. (1926). Protozoology. vol. 1, p. 552. London.

A NOTE UPON MYIASIS DUE TO THE LARVAE OF *CORDYLOBIA RODHAINI* GEDOELST

BY

D. S. BERTRAM

(*From the Department of Entomology, Liverpool School of Tropical Medicine*)

(*Received for publication November 3rd*, 1938)

The following note concerns the result of examining certain Diptera larvae sent from Mamfe, Cameroons. The writer is indebted to Professor D. B. Blacklock for supplying him with the material and with all the available information about the case.

The specimens were 4 of 16 larvae taken from discharging pustules on the neck and left arm of a man of 51 years of age. The case-history was ill-defined, but there was some indication that a wide-spread myiasis condition due to these larvae existed. The specimens have been identified as third instar larvae of *Cordylobia rodhaini* Gedoelst.

According to Broden and Rodhain (1910) parasitization of man by *C. rodhaini* is infrequent and accidental, and usually only one or two larvae occur in an infected person. The maximum number of larvae taken by them from a patient was 15. They have seen cases of both Europeans and natives, infection occurring on the scalp, below the lower eyelid, and on different parts of the trunk and limbs.

The distribution of the species is generally noted as being restricted to the forest-regions of equatorial Africa, particularly in the Belgian Congo. The literature, however, shows that records exist which indicate that this species is perhaps widely distributed in Africa.

Gedoelst (1905) first described the larva from a single specimen taken from the arm of a man, probably in the Belgian Congo. Later, in 1910, he described the second and third larval stages and a female from material received from Leopoldville, Belgian Congo. Broden and Rodhain (1910) recorded and described human myiasis in that district. Surcouf (1914) captured a male at Bas Ogoué, near Cap Lopez, French Equatorial Africa, and Gamble (1914) recorded the species from San Salvador, Angola. Rodhain and Bequaert (1916) found that myiasis, due to *C. rodhaini*, was common in antelopes (*Cephalophus dorsalis* Gr. and *C. grimmi* L.) and in the rodent, *Cricetomys gambianus* Wath., in the forest-regions north and south of the Uele river, Belgian Congo.

Also, a single larva was taken from a rat, *Rhynchocyon cirnei* Peters, but no cases in man were found in these regions. At Stanley Pool, it appeared that *C. gambianus* was the principal host. They identified one larva from Brazzaville, which is just within the French Equatorial African border at Stanley Pool. Macfie (1917) reported the occurrence of one larva in the forearm of a native at Accra, Gold Coast, and Loveridge (1923) obtained larvae from *Lophuromys a. aquilus* True at Bagilo, Tanganyika Territory. Bequart (1930) published a record of two larvae from a squirrel, *Heliosciurus punctatus* Temminck, at Oguma, French Congo, and for the Belgian Congo he noted larvae from *C. aequatorialis* Matschie at Bolobo and Barumbu, and from *Lasiopyga* (*Cercopithecus*) *wolfi* (Meyer) at Bikoro. *Cordylobia* larvae were not found in Liberia. In 1932, Symes and Roberts reported myiasis in the arm of a European near Ol Donyo Sabuk, Kenya, and Jack (1937) recorded from the Salisbury region of Southern Rhodesia a case of myiasis in a child. The present note establishes a record for the species in the British Mandated Cameroons.

It is perhaps worth while to note the following reports, which at one time were considered as possible records for *C. rodhaini*: myiasis in man in German East Africa (Skrozki, 1908); human myiasis in the region of the Ubangi river, Belgian Congo (Broden and Rodhain, 1910); a note upon an ovipositing female in the Transvaal (Pelser-Berensberg, 1911); four cases of human myiasis in Uala and Ngoko in French Equatorial Africa (Heckenroth and Blanchard, 1913); and myiasis in man in the area of the Ikelemba river, Belgian Congo, reported to Rodhain and Bequaert (1916).

It is well known that the larvae of *C. anthropophaga* Grünberg occur in man and in a wide range of animal hosts, and we conclude this note by indicating the literature available for comparison of *C. anthropophaga* and *C. rodhaini*. In addition, we have prepared from the material at our disposal a comparative note and figures of the characters which were found most useful in differentiating the third stage larva of the two species.

Descriptions of the larval stages and of the adults of *C. rodhaini* are given by Rodhain and Bequaert (1916), and of *C. anthropophaga* by Austen (1908) and Blacklock and Thompson (1923).

The generic characters of the genus *Cordylobia* Grünberg have been re-defined by Patton (1936), from whom we quote the following section concerning the larvae. ' LARVA. Cutaneous parasite of mammals, the first stage larva being equipped for entering the unbroken skin. THIRD STAGE LARVA. Cuticle armed with spines arranged irregularly on the thoracic and abdominal segments. Oral hooks of equal length, each with a lateral toothed plate. Anterior spiracles consisting of membraneous stalks bearing a number of short finger-like processes, each with an opening. Eighth segment rounded. Posterior spiracles small, not widely separated, peritreme mainly membraneous, the three breathing slits either short and straight, or longer and sinuous, and directed downwards and outwards.'

A COMPARISON OF THE THIRD STAGE LARVAE OF *C. ANTHROPOPHAGA* AND *C. RODHAINI*

C. anthropophaga. Spines small and often grouped in transverse rows of three or more spines (fig. 1, A); without a bar of spiny processes between the oral hooks; toothed plate bearing about six small yellow teeth (fig. 2, A); posterior spiracle with three short, slightly sinuous slits (fig. 2, B).

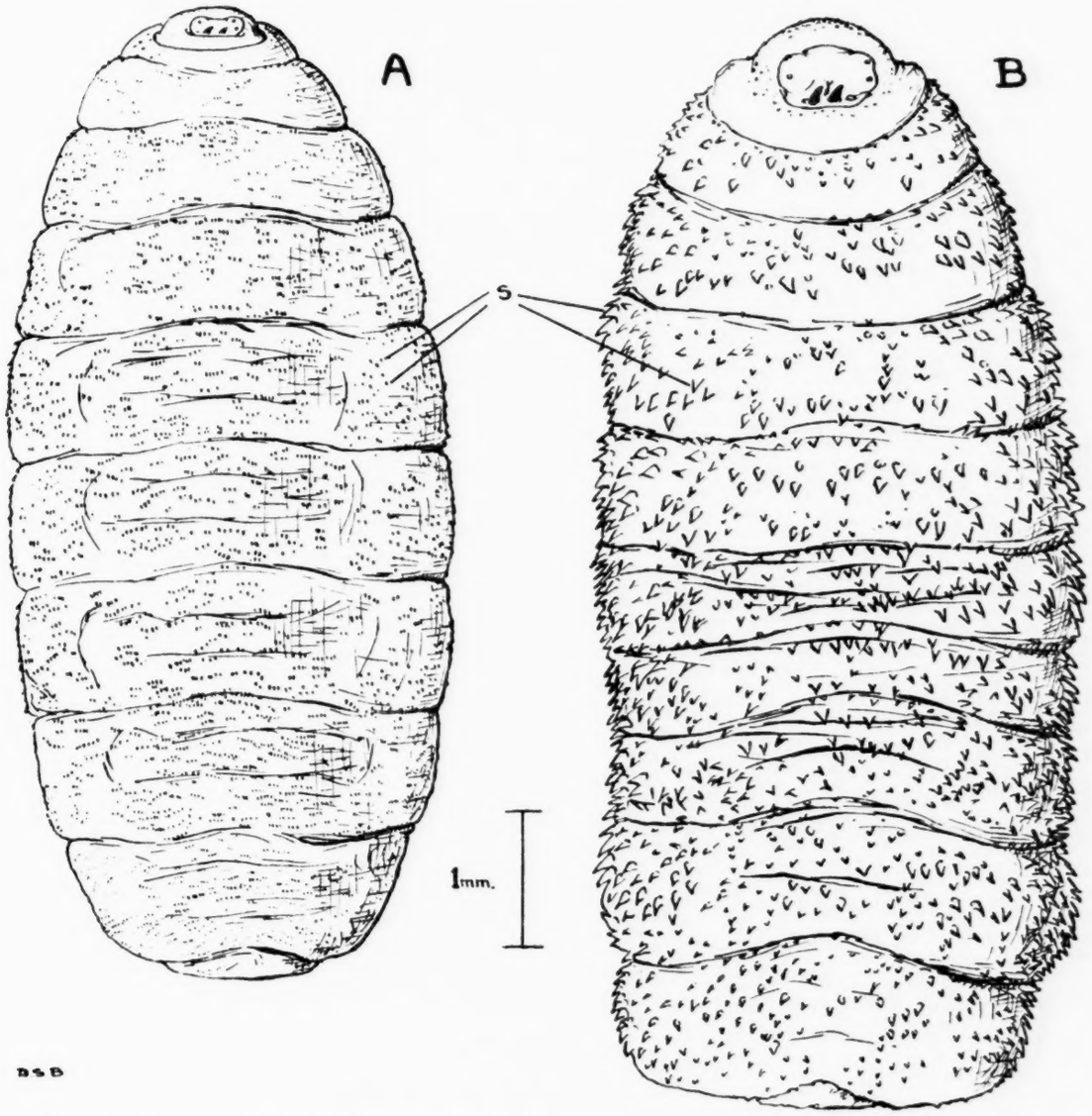


FIG. 1. A.—*C. anthropophaga*: third stage larva, ventral aspect. B.—*C. rodhaini*: third stage larva, ventral aspect. s.—Spines.

C. rodhaini. Spines (fig. 2, E, F) large and not grouped in transverse rows (fig. 1, B); with a dark bar of spiny processes between the oral hooks; toothed plate bearing about 10 stout yellow-brown teeth disposed in two rows (fig. 2, C); posterior spiracle larger than in *C. anthropophaga* and with three long tortuous slits, of which at least one may show fragmentation into two parts (fig. 2, D).

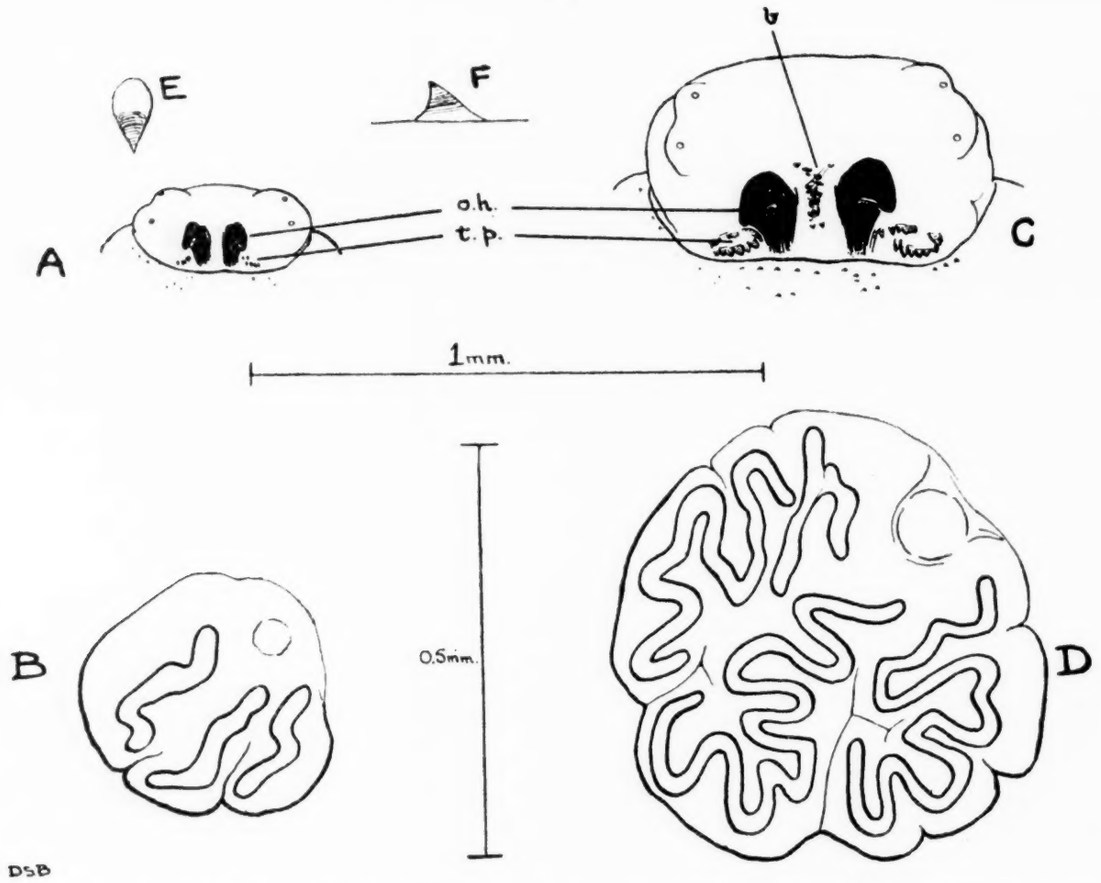


FIG. 2. A and B from third stage larva of *C. anthropophaga*. A.—Head segment, ventral aspect; B.—Left posterior spiracle.

C, D, E, F from third stage larva of *C. rodhaini*. C.—Head segment, ventral aspect; D.—Left posterior spiracle; E.—Body spine, optical section; F.—Body spine, side view. b.—Bar of spiny processes; o.h.—Oral hooks; t.p.—Toothed plate.

REFERENCES

AUSTEN, E. E. (1908). The tumbu-fly (*Cordylobia anthropophaga*, Grünberg). *Jl. Roy. Army Med. Corps.*, **10**, 18.

BEQUAERT, J. (1930). Medical and economic entomology. (Strong, R. P., Ed.: 'The African Republic of Liberia and the Belgian Congo,' vol. 2, chap. 36, p. 974. Cambridge, Mass.)

BLACKLOCK, B., and THOMPSON, M. G. (1923). A study of the tumbu-fly, *Cordylobia anthropophaga* Grünberg, in Sierra Leone. *Ann. Trop. Med. & Parasitol.*, **17**, 443.

BRODEN, A., and RODHAIN, J. (1910). La myiase cutanée chez l'homme au Congo. *Arch. Parasitol.*, **13**, 548.

GAMBLE, M. (1914). A list of blood-sucking arthropods from the Lower Congo, with a vocabulary. *Jl. Trop. Med. & Hyg.*, **17**, 148.

GEDOELST, L. (1905). Contribution à l'étude des larves cuticoles de Muscides africaines. *Arch. Parisitol.*, **9**, 568.

——— (1910). *Cordylobia rodhaini* nova species, Diptère congolais à larve cuticole. *Ibid.*, **13**, 538.

HECKENROTH, F., and BLANCHARD, M. (1913). Note sur la présence et l'endémicité d'une myiase furonculeuse au Congo français. *Bull. Soc. Path. Exot.*, **6**, 350.

JACK, R. W. (1937). Division of entomology: annual report for year 1936. *Rhod. Agric. Jl.*, **34**, 570; also *Bull. Minist. Agric., S. Rhodesia*, no. 1037.

LOVERIDGE, A. (1923). Notes on East African mammals collected 1920-1923. *Proc. Zool. Soc. Lond.*, p. 703.

MACFIE, J. W. S. (1917). The identifications of insects collected at Accra during the year 1916, and other entomological notes. *Rep. Accra Lab. for* 1916, p. 67.

PATTON, W. S. (1936). Studies on the higher Diptera of medical and veterinary importance : a revision of the genera of the family Muscidae Testaceae Robineau-Desvoidy based on a comparative study of the male and female terminalia : the genus *Cordylobia* Grünberg (sens. lat.). *Ann. Trop. Med. & Parasitol.*, **30**, 57.

PELSER-BERENSBERG, H. VON (1911). Notizen über *Cordylobia rodhaini*. *Soc. Ent.*, **26**, 34. (Reviewed by Rodhain and Bequaert, 1916.)

RODHAIN, J., and BEQUAERT, J. (1916). Matériaux pour une étude monographique des Diptères parasites de l'Afrique. II : Les myiases cutanées produites par *Stasisia* (*Cordylobia*) *rodhaini* Gedoelst. *Bull. Sci. France et Belg.*, **49**, 262.

SKROZKI (1908). Fliegenlarven in der menschlichen Haut. *Arch. f. Schiffs- u. Trop.-Hyg.*, **12**, 441.

SURCOUF, J. M. R. (1914). Note sur *Stasisia rodhaini* Gedoelst. *Rev. Zool. Afr.*, **3**, 475.

SYMES, C. B., and ROBERTS, J. I. (1932). A list of the *Muscidae* and *Oestridae* causing myiasis in man and animals in Kenya, recorded at the Medical Research Laboratory, Nairobi. *E. Afr. Med. Jl.*, **9**, 18.

A RECORD OF SOME COMPLICATIONS WHICH OCCURRED IN THE COURSE OF EXPERIMENTAL INFECTIONS OF AFRICAN VOLUNTEERS WITH *TRYPANOSOMA RHODESIENSE*

BY

J. F. CORSON

(*Medical Officer at Tinde Laboratory, Tanganyika Territory*)

(*Received for publication November 5th*, 1938)

It will be generally agreed that unsophisticated African volunteers should not be used for experimental infection with *Trypanosoma rhodesiense* unless the experimenter is convinced, on good grounds, that the infection will be free from risk of permanent injury to the health of the volunteers. Natives can at least understand, even if they cannot estimate, such risks as hunting dangerous animals or swimming flooded rivers, which they may undertake in the service of white people; these are risks which form part of their ordinary life. As regards infection with *T. rhodesiense*, the volunteers mentioned in this record had seen others resist infection or recover from it, or had been told about it by them; they knew that the bite of the infective fly might be followed by local swelling and by fever, and they accepted my assurance, or that of former volunteers, that the experiment was safe. They had sufficient intelligence and experience of Europeans to believe that such an experiment would not be made without a sure remedy for the disease. In fact, it may be said that they had some evidence and knowledge to guide them in deciding to offer themselves as volunteers.

From our experience of the action of germanin (Bayer 205), we have good reason to believe that it will make the experiment safe in every case of infection with *T. rhodesiense*; I know of no case where germanin failed to cause the trypanosomes to disappear from the blood, and when it is given immediately after the trypanosomes have been found in the blood, as in these experiments, it seems practically certain that it will cure the infection promptly. Van Hoof, Henrard and Peel (1938) refer to an exceptional case of infection of a man with *T. gambiense*, which was observed by Van den Branden and Van Hoof in 1924, where several doses of 1 gm. of germanin failed to clear the blood of trypanosomes; and I have seen advanced cases of Rhodesian sleeping sickness in which a temporary parasitic blood relapse occurred a few days after two or three doses of 1 gm. of germanin had been given (Corson, 1931). The thought that there might be an exceptional person whose experimental infection would not respond to treatment with germanin would cause more anxiety if we had not the experience of its action in thousands of cases.

A greater source of anxiety is the question of how long it is safe to wait before giving treatment to a volunteer who has apparently resisted infection ; if the incubation period were very long, or if an inapparent infection—cryptic, as it is sometimes termed—occurred, it is hypothetically possible that the central nervous system might have become involved before the infection was discovered. We have no knowledge of such an occurrence. In some unpublished experiments with *Cercopithecus* monkeys, which are very susceptible to infection with *T. rhodesiense*, I have found living trypanosomes in the cerebrospinal fluid as early as, but not earlier than, the tenth day after the infective *Glossina morsitans* had bitten the monkey ; in one case the trypanosomes infected a subinoculated rat. Keevill's cases of early infection in man have often been quoted (Keevill, 1928) ; they were all cured with germanin. I have seen one case of accidental infection of a European where the incubation period was one month, owing, no doubt, to very few trypanosomes having been injected by the fly ; treatment with germanin was given at once, and lumbar puncture, performed two days after the second dose, showed normal cerebrospinal fluid. The man was well four years later. In one of the cases recorded here the incubation period was 27 days (Case 9).

Germanin has an injurious action on the kidneys, causing albuminuria and the appearance of renal casts in the urine. This effect is much more pronounced in some Africans than in others, but in all cases it seems to be only temporary, the urine becoming normal again within a few weeks. It is, however, a disturbing complication when it occurs early during treatment.

Other sources of anxiety in experimental infections of African volunteers with *T. rhodesiense* are especially associated with the conditions and mode of life of Africans, or at least of such Africans as have volunteered at Tinde Laboratory, and, I think, of such as would be found in most places near areas of Rhodesian sleeping sickness, where *Glossina morsitans* and wild-game animals live. They are the presence of other diseases such as malaria, relapsing fever, plague, typhoid fever, typhus fever, cerebrospinal fever, filariasis, schistosomiasis, gonorrhoea and syphilis and, perhaps, small-pox. Fortunately yellow fever has not to be considered in Tanganyika Territory. It is necessary, therefore, to consider carefully whether the importance of the experiments justifies the acceptance of these volunteers.

There are various difficulties in making such experiments in towns, where, it might be thought, the general health of the natives would be better and they themselves more suitable as volunteers. Even in the large towns several diseases are very common, e.g., malaria, relapsing fever, schistosomiasis, filariasis, ancylostomiasis and venereal diseases, and such towns have a large floating population. There are also the difficulties of access to cases of sleeping sickness and the collection of wild animals of the kinds which occur in sleeping sickness areas. It is also probably desirable that the tsetse-flies should live in a climate similar to that of their natural habitat. Nevertheless, these difficulties may not

be insuperable, and it may be feasible to conduct such experiments in places that are largely free from the drawbacks mentioned, though at greater cost.

I have to acknowledge the offers of a few European men and women to be volunteers ; it could not reasonably be expected, however, that many European residents in tropical Africa would be sufficiently interested or sufficiently free to interrupt their duties and to allow themselves to undergo the discomfort of an attack of sleeping sickness and its treatment. It seems, then, that when the experiments are sufficiently important, the use of African volunteers is justified.

In this paper are recorded some of the complications that occurred in the course of experimental exposure to infection of 43 African volunteers during 1937 and 1938 ; of these, 28 became infected and 15 resisted infection. Each volunteer, except nos. 8, 9 and 10, was exposed to infection by the bite of a single infective *Glossina morsitans*. All were treated, whether infected or not, and germanin alone was used, in 1 gm. doses, given intravenously.

It was thought unwise, and was found to be unnecessary, to use any persuasion or any form of propaganda to induce volunteers to present themselves, and during 1938 about half of them were refused, chiefly on account of infection with urinary schistosomiasis ; a relatively large reward of money, however, was offered and their motive in volunteering was, of course, to gain it. They showed and expressed pleasure when an official appreciation of their action was conveyed to them.

In the following notes the numbers 1 to 43 refer to the volunteers in their chronological order ; albuminuria is shown as fractions representing the maximum proportionate height of the precipitate in the test-tube after boiling the urine, adding acetic acid, and allowing to stand for 24 hours.

1. Infected ; no complications.

2. Resisted ; albuminuria more than 1/2 after 2nd dose ; malaria after 7 months ; relapsing fever after 11 months ; malaria again after 14 months.

3. Infected ; 7 months later complained of fever at night, of weakness and wasting of the muscles ; lumbar puncture showed normal cerebrospinal fluid, and weekly weighing showed no loss of weight ; the illness was probably imaginary.

4. Resisted ; later found to have urinary schistosomiasis.

5. Infected ; albuminuria 1/3 after 3rd dose.

6. Infected ; had an undiagnosed fever 2 weeks after 4th dose ; malarial fever a month later.

7. Infected ; albuminuria 1/3 after 4th dose.

8. Inoculated ; resisted ; albuminuria 1/5 after 3rd dose.

9. Inoculated ; infected. This volunteer, like nos. 8 and 10, was inoculated with trypanosomes which had been transmitted to a monkey by *Glossina brevipalpis* in an experiment by Mr. Eric Burtt, which has been reported but is not yet published. As wild flies were used to transmit the Tinde strain of *T. rhodesiense*, the possibility of transmission of *T. brucei* could not be excluded, and so

tests on man were needed. The incubation period was 27 days, and the first dose of germanin was given on the 33rd day after inoculation. The case was peculiar, as trypanosomes were not found in blood-films taken from the ear, but a swelling appeared on his arm at the site of inoculation on the 27th day, and on the 31st day he complained of malaise and trypanosomes were found in blood-films taken from the arm swelling. He had no fever. White rats, subinoculated with blood from an arm-vein, did not become infected. I regarded him as having been infected with the strain of *T. rhodesiense* that was used in the experiment. Albuminuria 1/4 after 3rd dose; 4 months later headache and bodily pain relieved by or after quinine, but no parasites found in the blood; 2 months after this, he complained of fever and headache; temperature 99° to 100° F. Lumbar puncture showed a clear fluid, cells 5, 6 and 7 per c.mm., protein by Sicard and Cantaloube method 0·027 per cent. His temperature rose to 102° F., and a day or two later *Borrelia duttoni* was found in his blood.

10. Inoculated; resisted; malarial fever 4 months after 4th dose.

11. Infected; albuminuria 1/2 after 3rd dose; a month later he had gonorrhoea and 4 months later secondary syphilis.

12. Infected; albuminuria 1/7.

13. Infected; albuminuria 1/3.

14. Infected; albuminuria 1/8.

15. Infected; albuminuria 1/6.

16. Infected; albuminuria 1/4.

17. Infected; fainted just after first dose and had violent diarrhoea, probably caused by unsuitable food; albuminuria 1/3 after 2nd dose, 1/2 after 3rd, and 1/3 after 4th, but he was found to have urinary schistosomiasis.

18. Infected; albuminuria 1/6.

19. Infected; found later to have urinary schistosomiasis.

20. Infected; albuminuria 1/5 after 3rd dose; 3 months later developed two large intramuscular abscesses.

21. Resisted; a few days after the fly bit him he collapsed on the way to the laboratory and was brought in by car; recovered next day, and no signs of disease were found; illness perhaps imaginary; albuminuria 1/4 after 3rd dose; 2 months later brought in with fever and headache, temperature 101·6° F.; blood-films negative, and he was well next day.

22. Infected; no complications.

23. Resisted; headache and fever 10 days after the fly bit him; temperature 103·5° F. lasting one day only; blood examinations negative; albuminuria 1/3 after 3rd dose.

24. Infected; no complications.

25. Resisted; albuminuria 1/3 after 3rd dose.

26. Infected; no complications.

27. Resisted; headache and general pains 12 days after the fly bit him, and he was brought in by car and kept one day; no fever, and blood-films

negative. Six days after 2nd dose reported ill and brought in by car, temperature 102·6° F.; fever lasted 4 days, but blood-films negative; albuminuria 1/2 and numerous renal casts ; had diarrhoea after 3rd dose and headache and pains in feet after 4th dose.

28. Infected ; later found to have urinary schistosomiasis.

29. Resisted ; albuminuria 1/8 after 3rd dose.

30. Infected ; albuminuria 1/2 after 3rd dose.

31. Resisted ; albuminuria 1/10 after 3rd dose. Six weeks after the 4th dose had pain in left hip and thigh ; given aspirin powders to take at home ; no further report until 3 months later, when I was called out to him and found him greatly emaciated, with a pelvic abscess discharging in the left groin ; brought in and treated ; another abscess over Gluteus medius incised ; recovered after 2 weeks.

32. Infected ; 4 days after 3rd dose he showed haematochyluria, and microfilariae were found in the urine and blood ; he had had a similar attack many years before.

33. Infected ; no complications.

34. Infected ; on the evening of the day of his 3rd dose he developed fever and was brought in by car. His fever lasted for a week, the temperature varying between 102° F. and 105° F. but influenced by antipyretics ; he vomited several times during the first few days, and a morbilliform skin rash appeared on the 4th day and lasted for a few days. No parasites were found in his blood ; his blood serum was sent to Dar es Salaam Laboratory and was reported to agglutinate *B. typhosus* 1 : 50 and not *B. paratyphosus* Brucella or *B. proteus* X19. He recovered, and had no further complications.

35. Resisted ; had to be brought in one day for epigastric pain.

36. Resisted; on the 4th day after the fly bit him, he had fever, with temperature of 100·2° F., and the next day microfilariae were found in his blood ; remained 3 days and was then taken home. Complained of diarrhoea after first dose of germanin, and after the 3rd dose developed fever, with temperature 103·6° F., which lasted 4 days and was accompanied by pains in the body and right foot ; no cause of the fever was found.

37. Resisted ; albuminuria 1/2 after 2nd dose ; 5 days after the 2nd dose reported to be very ill and was visited and brought in ; he was very collapsed and had apparently had severe vomiting and diarrhoea ; recovered after 4 days. Five months later had intramuscular abscess of right posterior axillary fold, which quickly healed after incision.

38. Infected ; albuminuria 1/6 after 3rd dose.

39. Infected ; albuminuria 1/6 after 3rd dose. Seven weeks after the 4th dose complained of tinnitus, especially in the left ear ; there was no pain. A month later he returned, saying that he was very ill and had very bad tinnitus in the left ear. Kept at the laboratory for observation ; said that he had had tinnitus for the last fortnight, had pain in arms and legs, and felt weak. There

was no fever, blood examinations were negative, and he ate and slept well. Lumbar puncture was not done. Two days later he said that he felt better and asked to return home; he was motored to near his house, about two miles from the laboratory, and told to send word if he felt ill again. A month later an old man, who shared his hut, arrived with the news that he had died the previous morning and was buried; he had been very ill since 4 days after his return home; he had not sent word to the laboratory, but had informed the headman, as that, the old man said, was their custom.

40. Resisted; albuminuria 1/8. Six days after 2nd dose complained of pains in legs and feet; kept at the laboratory for 2 days; no fever, and blood examinations negative. On the day after 4th dose complained of pains in head and limbs. No further complications.

41. Resisted; a slight attack of malaria after 4th dose.

42. Infected; albuminuria 1/4 after 3rd dose.

43. Infected; on the day after his 3rd dose complained of burning pains in both feet, which continued for 6 days; on the 7th day had little pain and received his 4th dose. Two days after this I was called out to him and found him complaining of very severe pains in the feet, and brought him in by car; his temperature was normal. Next day he felt rather better, but his feet were still painful; on the following day developed fever, with temperature 103·6° F., and complained of very severe headache and pain in the feet; blood showed microfilariae; his temperature remained high all day and his headache was only relieved by opium. On the following day he seemed to be very ill; his temperature at 5 a.m. was 103·6° F. and his pulse was 140; complained of defective vision, very severe headache and pain in the feet. Lumbar puncture was done with a fine needle and the fluid came out in a jet; it was clear, there were 5 and 0 leucocytes in two successive c.mm., and the amount of protein, estimated by Sicard and Cantaloube's method, was 0·027 per cent. Two c.cm. of the fluid were inoculated into a white rat, and the rat showed no infection. Stained smears of the cerebrospinal fluid showed neither cells nor micro-organisms. His temperature remained high, rising to 105° F., but it fell to 101° F. on the 4th day and was normal on the 5th day. He developed a severe necrotic stomatitis and tonsillitis, but recovered after some days and had no further complications.

There was no reason to think that either the infections with trypanosomes or the treatment with germanin had anything to do with the complications, except with the albuminuria. At one period, instead of the ordinary freshly distilled water, double distilled water in ampoules was used to prepare the solution of germanin, but there seemed to be no connection between symptoms such as abdominal pain and leg pains and the water used for the injections. Some volunteers, with the same treatment, had no symptoms. There seems to be no reason to think that these intercurrent illnesses were exceptional among these people, who are exposed to many parasitic diseases, have primitive ideas

about health and sanitation, and are mostly careless and thoughtless. Puzzling illnesses are commonly ascribed to witchcraft. The more serious complications fortunately occurred after the 3rd or 4th dose of germanin had been given, so that the infection with trypanosomes could be regarded as cured before the complication appeared.

REFERENCES

CORSON, J. F. (1931). Further observations on the trypanocidal action of human blood serum on *Trypanosoma rhodesiense* in white rats. *Jl. Trop. Med. & Hyg.*, **34**, 81.

KEEVILL, A. J. (1928). *Trypanosoma rhodesiense* infection treated with 'Bayer 205' or 'Fourneau 309.' *Trans. Roy. Soc. Trop. Med. & Hyg.*, **22**, 83.

VAN HOOF, L., HENRARD, C., and PEEL, E. (1938). The stability of Bayer 205 resistance in *Trypanosoma gambiense*. *Ibid.*, **32**, 197.

THE MARY KINGSLEY MEDAL

This medal was struck in commemoration of the work of the late Miss Mary Kingsley in West Africa, and is conferred in recognition of distinguished scientific achievement.

HONORARY RECIPIENTS

Her Royal Highness Princess Christian
Lord Lister
The Right Hon. Joseph Chamberlain
Prince Auguste d'Arenberg
Mrs. Pinnock
William Adamson
William Carter
Miss A. R. Caton
Mrs. John Middlemass Hunt
Lady Danson

RECIPIENTS

1905—

Colonel Sir David Bruce
Robert Koch
A. Laveran
Sir Patrick Manson

1907—

B. Danilewsky
Charles Finlay
W. M. Haffkine
Camillo Golgi
Surgeon-General W. C. Gorgas
Theobald Smith

1910—

R. Blanchard
Anton Breinl
Angelo Celli
C. W. Daniels
Surgeon-General Sir Alfred Keogh
Colonel W. G. King
Sir William Macgregor
B. Nocht
G. H. F. Nuttall
Major Leonard Rogers
J. L. Todd
Surgeon-General Walter Wyman

1913—

Frederick V. Theobald

1917—

Griffith Evans

1919—

J. W. Scott Macfie
The Oswaldo Cruz Institute, Rio de Janeiro

1920—

Major E. E. Austen
Sir Arthur G. Bagshawe
Sir Andrew Balfour
A. L. G. Broden
Mrs. Chalmers, in recognition of the work of the late Dr. A. J. Chalmers
G. Grassi
R. T. Leiper
F. Mesnil
Edmond Sergent
C. W. Stiles
T. Zammit

1929—

G. Carmichael Low
G. A. K. Marshall
Robert Newstead
A. T. Stanton
J. W. W. Stephens
C. M. Wenyon

1934—

Henry Beeuwkes
Sir George Seaton Buchanan
Sir S. Rickard Christophers
Sir Malcolm Watson

1938—

Marshall Albert Barber
Emile Brumpt
Walter Scott Patton
Werner Schulemann

PRESENTATION OF MARY KINGSLEY MEDALS

On the evening of Wednesday, November 23rd, 1938, the fortieth anniversary of the founding of the Liverpool School of Tropical Medicine, the Mary Kingsley medal, which is the School's highest award, was presented to one honorary recipient and to four distinguished scientists who have done much notable work in tropical medicine. The Viscount Leverhulme, chairman of the School, entertained the medallists and others to dinner at the University Club, and afterwards held a reception at the School of Tropical Medicine, where he presented the medals in the presence of a large gathering of distinguished guests.

In his introductory remarks before the presentation of the medals, Lord Leverhulme said :

' I would like, as chairman of the Liverpool School of Tropical Medicine, to welcome all the guests, more especially those who are to receive the Mary Kingsley medal.

' This is the second time I have been privileged, on behalf of the Council, to present these medals, which are the highest distinction which the School can bestow.

' On the last occasion, in 1934, I had the honour of reading a message from His Royal Highness the Duke of York, who was then our President. After ascending the throne, our former President honoured the School by graciously accepting the office of Patron. At the Annual Meeting this afternoon, a telegram of loyal wishes and dutiful appreciation was sent to the King, and the following gracious reply has been received :

> ' " I sincerely thank the Council and friends of the Liverpool School of Tropical Medicine for their kind and loyal message. As Patron, I am glad to know of the presentations of medals which are being made this evening and assure you of my continued interest in the work of the School.
>
> ' " GEORGE R.I."

' I am sure that all associated with the School are gratified to have this token of His Majesty's continuing interest in its work.

' When the office of President became vacant, as I told the Annual Meeting this afternoon, there seemed to be only one person who could be first asked to fill it—Lord Derby—and he most kindly consented to do so.

' The Mary Kingsley medal was founded in 1904 to commemorate the work of Mary Kingsley—whose uncle was Charles Kingsley the novelist—in promoting the welfare of the natives of West Africa. Mary Kingsley had personal associations with Liverpool, so it is most appropriate that this distinction should be linked with her name. The qualifications for a recipient of the medal are services in the cause of preventing and fighting disease in the tropics. There have been many distinguished recipients in the past, and the standard is well maintained by those whom we are honouring to-night. When introducing them, Professor Warrington Yorke will speak with more authority and in greater

detail about their scientific qualifications than I can, but I would like to suggest that their acceptance of this honour is yet another example of the international character of all true science. Dr. M. A. Barber is at present working in Mexico and so unfortunately cannot receive the award in person. In Emeritus Professor Patton we have one who was a Professor here for ten years and a distinguished British entomologist, while Professor Brumpt comes from Paris and Professor Schulemann from the University of Bonn in Germany. Finally there is the award which I feel sure will give the greatest possible pleasure to her friends in this neighbourhood, the one to Lady Danson, whose late husband was chairman of the School and one of its founders. It has become customary, when awarding these medals, from time to time to step outside the ranks of scientific workers and honour the names of those who, although not technologists, will always remain associated with the progress of tropical medicine. We have the happiest memories of the late Sir Francis Danson, whose son is a member of our Council, and I am sure you will agree that Lady Danson is a most fitting recipient of a Mary Kingsley medal.

'A little while ago, I happened to see a performance of a one-act play by Neil Grant, in which one of the characters is a microbe. The play is called "The Last War" and suggests that, in the final Armageddon, when the warring nations are busy cancelling each other out, after bullet, shell, bomb and gas have done their worst, the entomologist will be harnessed to the chariot of war and the parasitologist will put on the armour of Mars in order to complete the holocaust. The play depicts a group of animals discussing the situation after the last specimen of *Homo sapiens* has been wiped out. Actually, in the play, one soldier escapes, but that is by the way. The play gives rise to awesome thoughts, for one certainly feels that, however much scientists in other fields may turn their attention to new means of destruction, medicine in general and tropical medicine in particular will always be devoted to the saving of life only.

'It is an interesting thesis, but meanwhile the School continues on its beneficent way. I can hardly believe that Professor Warrington Yorke, even if he were willing to try, could call a halt to his long campaign against the bacillus, and persuade it to be his ally instead of his enemy, or that Professor Gordon, even with all the resources of the insectarium, could raise a race of microbes which would obligingly take sides in a world war. Forgive this digression!

'Before calling upon Professor Warrington Yorke, I would like to call your attention to two significant anniversaries. This year marks the fortieth anniversary of the founding of this School, and it is just half a century ago that the late Sir Ronald Ross made the great discovery of the transmission of malaria by the mosquito. To realize what that means, we have to try to imagine what the tropics would be to-day if the discovery had never been made; what life there would be like if, although doctors might know how to treat and cure malaria,

they had no idea how it arose and no knowledge of what sanitary precautions had to be taken to prevent it. It was an epoch-making discovery, and this School can be proud to recall that Ross became a member of the original staff here and accompanied a succession of expeditions sent by the School to various tropical regions.'

Professor Warrington Yorke then presented the five recipients in the following terms :

1. Lady Danson. ' The first Mary Kingsley medallist on this occasion is a lady distinguished in many ways and well known to us all. By this award we convey our appreciation of the part which she has taken, and is still taking, in promoting the investigation of tropical diseases, thus furthering the endeavour of her late husband.

' The association of Sir Francis Danson with this Institution began very early in its history. As you have heard, the School was founded in the year 1898 ; and already, in 1901, we discover Mr. Danson representing the Council of the University on the School Committee. In 1908 he attained the office of vice-chairman, and in 1913 he succeeded the late Lord Leverhulme as chairman of the School, a post which he continued to hold until his death in 1926.

' It was during his lengthy period of office as chairman that he saw the establishment of the School's research laboratory at Freetown, in Sierra Leone, West Africa, for the construction of which a sum of £10,000 had been bequeathed by the founder of the School.

' Sir Francis Danson took a deep interest in the opening of this West African laboratory, and, while he fully recognized the strain which this scheme must throw upon the limited financial resources of the School, he felt so optimistic about the prospects of research there that he urged the professional staff and Committee to risk the venture, leaving for the future the question of adequate endowment of the laboratory. From the scientific point of view his optimism has been justified. The Sir Alfred Lewis Jones Laboratory in Freetown is an institution which has made valuable contributions towards the solution of many problems of tropical disease.

' In the year 1920, Mr. Danson's services to this School and to other public bodies in Liverpool were rewarded by the conferment of a knighthood. How great a part he played in the life of Liverpool could only be gauged from a full recital of the number of important offices he held—a recital too long for an occasion such as this.

' But Lady Danson was far from idle during all the years in which her husband presided over the destinies of the School. She was not content to be a passive spectator of its rapid development, but took the keenest interest in all its varied and sometimes disconcerting phases. On occasion, during her husband's enforced absence owing to sickness, she found herself suddenly called upon to assume, at public functions, the duties of chairman. It was at such times that she revealed, somewhat to the surprise and greatly to the

enlightenment of members of the Committee and of the professional staff, not only that she had a thorough grasp of the affairs of the School, but also, incidentally, that she herself was no mean orator.

'It is, therefore, no less on account of her own faith in the future of the School than in recognition of her late husband's long services to it that we have particular pleasure in awarding the Mary Kingsley medal to Lady Danson.

2. 'Seventy years ago—to be precise, 70 years ago yesterday—DR. MARSHALL ALBERT BARBER was born at Crown Point, in India. I suppose that, like other children born in India, he was tormented by mosquitoes. If this be so, he has exacted a full revenge from these enemies of mankind, for during the past quarter of a century he has devoted himself to the study of their habits, and has added greatly to the knowledge of how best they may be destroyed.

'Amongst his many contributions to this branch of science, there ranks foremost his discovery of the manner in which "Paris green," an arsenic compound, can be used as an effective and inexpensive means of destroying the larvae of malaria-carrying mosquitoes—a discovery which has benefited mankind in every part of the world where malaria afflicts the human race.

'Dr. Barber's long and distinguished association with the International Health Division of the Rockefeller Foundation is well known, not only on account of his researches in malaria, but also for his work on the hookworm. During his visit to the Orient in 1915-1917, Barber published papers on both these diseases, and showed clearly for the first time the part played by each in producing the anaemia which is so pronounced a feature of the inhabitants living in districts where the diseases are endemic.

'A man more versatile than most of his fellows, he has in his time laboured in many fields of science, for he was an Assistant in Botany at Harvard as long ago as 1892, and became Professor of Bacteriology and Pathology at Kansas at the early age of 26. It was during this period of his career that he invented the apparatus with which his name will always be associated, the "Barber micro-manipulator." This instrument, like so many great inventions, was simple in its original conception, but its introduction marked an important advance in medical science, for by its aid bacteriologists are now able to study the growth of individual minute organisms, and biologists to dissect individual cells, under far higher magnifications than were previously possible.

'Dr. Barber has had an exceptionally long and distinguished scientific career. Thirty-five years ago he published his first important contributions to medical science, and to-day, at 70 years of age, he still continues, with that indefatigable zeal which is so characteristic of him, to add to the store of human knowledge by his studies of malaria in India and America.

'We much regret that Dr. Barber, who is at present working in Mexico, is unable to be with us to-night; but we are pleased to welcome his distinguished colleague, Professor Gunn, vice-president of the Rockefeller Foundation, who has kindly consented to receive the medal for him.

3. ' We honour next Professor Brumpt. PROFESSOR EMILE BRUMPT was born in Paris in the year 1877. He studied at the Sorbonne and in the Faculty of Medicine of Paris, and was awarded the degree of Doctor of Natural Sciences in 1901. He became a Doctor of Medicine in the year 1906, presenting a thesis on mycetoma. In 1907 he was appointed Associate Professor of Parasitology and Natural History in the Faculty of Medicine, and in 1919 he attained full professorial status.

' It was in this year also that he was elected a member of the Academy of Medicine, and it was not long before he was being honoured by membership of numerous foreign scientific academies and societies. In the same year he undertook the heavy duties of Secretary General of the Institute of Colonial Medicine of France, which he still carries out with energy. In the year 1926 he was appointed Director of the School of Malariology of the University of Paris.

' Professor Brumpt is the author of over 350 published papers, covering a wide field of scientific investigation. One of his outstanding contributions to the wider dissemination of knowledge of the subject of parasitology was his foundation of the *Annales de Parasitologie Humaine et Comparée*, a journal which he has directed from its inception. His name is familiar to students in every country from the popularity which has been attained by his well-known text-book on parasitology, of which compendious tomes—there are two of them—the fifth edition has recently appeared.

' Brumpt, the professor, the scientific and successful investigator into innumerable problems of tropical parasitology, is a name to conjure with in every centre where research in parasitology is in progress. Brumpt, the man, is certainly one of the most familiar medical figures in the world to-day. He has for many years been an indefatigable traveller, and the celerity of his meteoric excursions over the surface of the globe is something quite remarkable. No form of transit is too rapid for him. The directors of railway, shipping and air-line companies must all bless the name of Brumpt—that is, of course, assuming that he is always a fare-paying passenger ! If one wishes to discover Brumpt, one may look for him almost anywhere in the wide world with an equal chance of success. He may be in China or Peru, on the Amazon or the Euphrates ; he may be sitting chilly on the Rocky Mountains or plunging in perspiring haste through the forests of equatorial Africa. Wherever he is found he will always be the same alert, earnest investigator. He is the personification of the ardent spirit of research, filling each " unforgiving minute with sixty seconds' worth of distance run."

4. ' In honouring EMERITUS PROFESSOR WALTER SCOTT PATTON we are honouring a colleague who has worked for ten years with us in this School.

' Patton was born in India in 1876, and, after obtaining his medical degree in Edinburgh and spending some time in postgraduate study in Germany,

he joined the Indian Medical Service in 1902 and returned to the land of his birth. Here for twenty years he laboured, adding much to our knowledge of the diseases of that country. The Indian Medical Service contains the names of many who have achieved fame in science, and Patton's occupies a place of honour amongst them. The routine duties of an officer in the Indian Medical Service are manifold and arduous, yet, like his comrades Ross and Leishman, Patton always found time—the time that most men would have devoted to recreation—to pursue his investigations. The value of his work was early recognized by his appointment as a specialist to investigate the aetiology of kala-azar and Oriental sore, and later by his promotion to the directorship of the King Institute of Preventive Medicine in Madras.

' In 1921, he joined the staff of the University of Edinburgh. He was not, however, allowed to remain long in this academic sanctuary, for in 1925 the Royal Society organized the Kala-Azar Commission to Northern China, and selected Patton to be its director.

' In 1927, Patton was appointed to the Dutton Memorial Chair of Entomology in this University. During the ten years which he has occupied this Chair his kindliness and courtesy endeared him to all with whom he came in contact ; and it was with the very deep regret of all his colleagues that it was learned in December last that he had been compelled to resign owing to ill health.

' With a modesty rare in this self-seeking age, Patton has been content to work long and arduously, seeking only to help his fellow men. In this country, in India and elsewhere there are colleagues who owe much to their association with him, and scattered over the world there is an army of students whom he taught with that patient care and attention to detail which are characteristic of the man. But there is another and far greater army who, though they have never met him, know him through his books. They are formidable, imposing volumes, these Debretts of the entomological world, whose pages you examine with the comforting thought that if it is not in " Patton " it is not worth while. Not, perhaps, books to be lightly read, but rather with the feeling that

> " Here index learning turns the student pale
> Yet holds the eel of science by the tail."

' We all regret that Professor Patton is unable to attend to-night to receive the medal in person. But he is represented by a colleague who has worked with him for 32 years—one who has rejoiced with him in his many successes and shared his few failures, and who has added much to the interest and scientific value of his numerous publications by those exquisite drawings which bear the well-known signature E.M.P. I refer, of course, to his wife, whose presence here to-night gives us all great pleasure.

5. ' PROFESSOR WERNER SCHULEMANN was born in Silesia in 1888, and was educated in the Universities of Freiburg and Breslau, where he studied

chemistry and medicine. He gained his degree in science in 1913, and graduated in medicine the following year. After the Great War he joined the great German chemical firm of Bayer, and soon rose to the directorship of that firm's celebrated pharmacological laboratories at Elberfeld. There he devoted his remarkable gifts to the study of pharmacology; he synthesized new chemical compounds which are now widely used as local anaesthetics and soporifics and for a variety of other medicinal purposes. His researches on the relationship between chemical constitution and pharmacological activity have received universal recognition.

'Although Schulemann has never visited the tropics, his researches at Elberfeld have resulted in a discovery of such importance to tropical medicine that his name is known to all workers in this field.

'In order that you may appreciate the full significance of Schulemann's work I must take you back to 1905, when the late Dr. Wolferstan Thomas of this School discovered that it was possible to cure animals infected experimentally with the sleeping sickness organisms by means of a practically unknown and unused proprietary substance called "atoxyl." This substance, which was soon shown to be a compound of arsenic and aniline, was immediately tried with striking success in cases of human sleeping sickness, which at that time was destroying hundreds of thousands of lives in tropical Africa, and for which we had no remedy.

'This empirical discovery—that it was possible to cure a fatal infection by a chemical compound manufactured in the laboratory—called forth Ehrlich's classical researches, which culminated a few years later in the production of salvarsan, or "606," as a cure for syphilis. The modern science of chemotherapy—the treatment of infectious diseases by chemical substances—was thus established on a sure foundation, and soon other compounds were prepared whereby many of the deadly diseases of the tropics can now be cured.

'The problem of malaria—the greatest of all tropical diseases—still remained. Fortunately, we were far from unarmed in our warfare on this disease because nature had provided a remarkable remedy in quinine. Nevertheless, the efforts of the chemist had failed to produce any drug which was of the slightest value in the treatment of malaria.

'In 1924, Schulemann published a discovery of the greatest importance. He had succeeded in synthesizing in the laboratory a chemical compound which not only acted on the malaria parasite, but accomplished what quinine had failed to do, in that it destroyed the stage of the parasite which infects the mosquito. This outstanding achievement attracted world-wide attention and gave a great impetus to the comparatively new science of chemotherapy, the full significance of which is only now beginning to be appreciated.

'Although Schulemann is the last of the distinguished scientists whom we are honouring to-night, he is certainly not the least. Beneath his formidable exterior is a genial and kindly nature which has made for him a host of friends

in many countries. A man of wide interests and accomplishments, he touches life at many points. He speaks four languages fluently, and has a profound admiration for all that is beautiful: to visit the galleries of Rome under his guidance is a delight.

'Two years ago Schulemann left the firm of Bayer to become Professor of Pharmacology at Bonn; he has the best wishes of all his friends in his endeavour to create at that ancient seat of learning a department worthy of the great subject which he adorns.'

PRESENTATION OF MARY KINGSLEY MEDALS

November 23rd, 1938

Standing (*left to right*) : Professor Brumpt ; Lord Leverhulme (Chairman of the School) ; Lady Danson ; Dr. A. D. McNair (Vice-Chancellor of the University) ; Professor Warrington Yorke.

Seated (*left to right*) : Professor Schulemann ; Mrs. Patton (who received the medal on behalf of Emeritus Professor W. S. Patton) ; Lady Leverhulme ; Mr. H. D. Dickie (Vice-Chairman of the School) ; Professor Gunn (who received the medal on behalf of Dr. M. A. Barber).

UNIV. OF MICH.

INDEX

INDEX

PAGE

INDEX TO AUTHORS xii
GENERAL INDEX..... xii
INDEX TO SPECIES NEW TO SCIENCE xviii

INDEX TO AUTHORS

PAGE

Bertram, D. S. 431
Binns, H. R. 425
Blacklock, D. B. 39, 109
Caballero y C., E. 225
Christophers, Sir S. R.; and Fulton, J. D. 43, 77, 257
Corkill, N. L. 333
Corson, J. F. 197, 245, 437
Daengsvang, S.; and Tansurat, P. 137
Davey, T. H.; and Gordon, R. M. 413
Devine, J. 163
Foy, H.; and Kondi, A. 249
Fulton, J. D.; and Christophers, Sir S. R. 43, 77, 257
Gibbins, E. G. 9, 21
Glyn-Hughes, F.; Lourie, E. M.; and Yorke, W. 103
Gordon, R. M.; and Davey, T. H. 413
Hawking, F. 313, 367, 383
Ho, C. 115, 279, 287, 387
Ho, C.; and Patton, W. S. 141
Kau, L. S.; and Wu, K. 129, 133
King, H.; Lourie, E. M.; and Yorke, W. 177

PAGE

Kirk, R. 339, 357
Kirshner, A. 159
Kirshner, A.; and Southwell, T. 95, 193
Kondi, A.; and Foy, H. 249
League of Nations 1
Lourie, E. M.; Glyn-Hughes, F.; and Yorke, W. 103
Lourie, E. M.; King, H.; and Yorke, W. 177
Lourie, E. M.; and Yorke, W. 201
Patton, W. S.; and Ho, C. 141
Pawan, J. L. 35
Pinkerton, H.; and Weinman, D. 215
Southwell, T.; and Kirshner, A. 95, 193
Tansurat, P.; and Daengsvang, S. 137
Walker, A. J. 231
Weinman, D.; and Pinkerton, H. 215
Wu, K.; and Kau, L. S. 129, 133
Yorke, W.; Glyn-Hughes, F.; and Lourie, E. M. 103
Yorke, W.; King, H.; and Lourie, E. M. 177
Yorke, W.; and Lourie, E. M. 201

GENERAL INDEX

PAGE

Abyssinian louse-borne relapsing fever, a laboratory study of 339
" louse-borne relapsing fever, the non-transmission of, by the tick *Ornithodorus savignyi* and certain other blood-sucking arthropods 357
Acriflavine, a quantitative study of the photosensitivity induced in trypanosomes by acriflavine 367

PAGE

Acriflavine and trivalent arsenicals, analysis of the trypanocidal action of 313
Anopheles, a new tree-hole breeding *Anopheles* from the island of Hainan 279
" *barbirostris* Van der Wulp ... 389
" *costalis*, fungal infections of mosquitoes, especially of *A. costalis* 231
" *hyrcanus* var. *nigerrimus* Giles 388

PAGE

Anopheles hyrcanus var. *sinensis* Wiedemann 388
„ *jeyporiensis* var. *candidiensis* Koidzumi 396
„ *kochi* Dönitz 391
„ *maculatus* var. *hanabusai* Yamada 402
„ *minimus* Theobald..... 393
„ *philippinensis* Ludlow 406
„ *sintonoides* sp. nov. 279
„ *subpictus* var. *indefinitus* Ludlow (?)..... 397
„ *vagus* Dönitz 398
Anopheline mosquitoes from the island of Hainan 387
Arcus senilis as a sign of chronic vitamin C deficiency 333
Arsenicals, trivalent, and acriflavine, analysis of the trypanocidal action of 313
Arthropods, the non-transmission of Abyssinian louse-borne relapsing fever by the tick *Ornithodorus savignyi* and certain other blood-sucking arthropods 357
Atebrin, a note on the trypanocidal action of atebrin in relation to its absorption by trypanosomes 383
„ observations on the course of *Plasmodium knowlesi* infection in monkeys, with notes on its treatment by atebrin 257
„ in treatment of malaria : study of the therapeutics and prophylaxis of malaria by synthetic drugs as compared with quinine 1
Bartonella of the guinea-pig, *Bartonella tyzzeri* sp. nov..... 215
Bartonella tyzzeri sp. nov..... 215
Bat, an unusual strain of rabies virus in 35
Bertram, D. S. A note upon myiasis due to the larvae of *Cordylobia rodhaini* Gedoelst 431
Binns, H. R. Observations on the behaviour in laboratory animals of *Trypanosoma congolense* Broden, 1904 425
Bird malaria, a note on the action of 1 : 11 normal undecane diamidine on..... 257
Blacklock, D. B. A device for applying oil or other liquids to flushing cisterns 109
Blacklock, D. B. Protection for the roofs of buildings in the tropics..... 39

PAGE

Blood, changes in, produced by the administration of undecane diamidine 163
Buildings in the tropics, protection for the roofs of 39
Caballero y C., E. Nematodes of the reptiles of Mexico, II..... 225
Cattle in China, a note on the pathology of schistosomiasis due to *S. japonicum* among 129
Cerebrospinal fluid of monkeys (*Cercopithecus* sp.) infected with a strain of *Trypanosoma rhodesiense*..... 197
Chemotherapy, studies in 103, 163, 177, 201
China, a note on the pathology of schistosomiasis due to *S. japonicum* among cattle in China.....129
Christophers, Sir S. R., and Fulton, J. D. The inhibitive effect of drugs upon oxygen uptake by trypanosomes (*Trypanosoma rhodesiense*) and malaria parasites (*Plasmodium knowlesi*) 77
Christophers, Sir S. R., and Fulton, J. D. Observations on the course of *Plasmodium knowlesi* infection in monkeys (*Macacus rhesus*), with notes on its treatment by (1) atebrin and (2) 1 : 11 normal undecane diamidine, together with a note on the action of the latter on bird malaria..... 257
Christophers, Sir S. R., and Fulton, J. D. Observations on the respiratory metabolism of malaria parasites and trypanosomes 43
Cisterns, a device for applying oil or other liquids to flushing cisterns 109
Coelomomyces africanus sp. nov..... 239
Cordylobia anthropophaga, a comparison of the third stage larva of, with that of *C. rodhaini* ... 433
„ *rodhaini* Gedoelst, a note upon myiasis due to the larvae of..... 431
Corkill, N. L. Arcus senilis as a sign of chronic vitamin C deficiency..... 333
Corson, J. F. The cerebrospinal fluid of monkeys (*Cercopithecus* sp.) infected with a strain of *Trypanosoma rhodesiense* 197
Corson, J. F. A record of some complications which occurred in the course of experimental infections of African volunteers with *Trypanosoma rhodesiense* 437

PAGE

Corson, J. F. A third note on a strain of *Trypanosoma gambiense* transmitted by *Glossina morsitans* 245

Daengsvang, S., and Tansurat, P. A contribution to the knowledge of the second intermediate hosts of *Gnathostoma spinigerum* Owen, 1836..... 137

Davey, T. H., and Gordon, R. M. The size of aperture necessary in screen-cloth intended for the protection of dwellings in West Africa 413

Devine, J. Studies in chemotherapy. XVIII : Changes in the blood and urine produced by administration of undecane diamidine..... 163

Drugs, inhibitive effect of drugs upon oxygen uptake by trypanosomes (*Trypanosoma rhodesiense*) and malaria parasites (*Plasmodium knowlesi*)..... 77

Ethiopian Simuliidae, notes on..... 21

Eumusca Townsend..... 303

Fasciolopsis buskii, pathological findings among pigs experimentally infected with 133

Female terminalia of house-flies as a grouping character, the significance of 287

Foy, H., and Kondi, A. A note on intracorpuscular methaemoglobin in plasmochin toxicity 249

Fulton, J. D., and Christophers, Sir S. R. The inhibitive effect of drugs upon oxygen uptake by trypanosomes (*Trypanosoma rhodesiense*) and malaria parasites (*Plasmodium knowlesi*)..... 77

Fulton, J. D., and Christophers, Sir S. R. Observations on the course of *Plasmodium knowlesi* infection in monkeys (*Macacus rhesus*), with notes on its treatment by (1) atebrin and (2) 1 : 11 normal undecane diamidine, together with a note on the action of the latter on bird malaria..... 257

Fulton, J. D., and Christophers, Sir S. R. Observations on the respiratory metabolism of malaria parasites and trypanosomes 43

Fungal infections of mosquitoes, especially of *Anopheles costalis* 231

PAGE

Gibbins, E. G. The mouth-parts of the female in *Simulium damnosum* Theobald, with special reference to the transmission of *Onchocerca volvulus* Leuckart 9

Gibbins, E. G. Notes on Ethiopian Simuliidae, II 21

Glossina morsitans, a third note on a strain of *Trypanosoma gambiense* transmitted by 245

Glyn-Hughes, F., Lourie, E. M., and Yorke, W. Studies in chemotherapy. XVII : The action of undecane diamidine in malaria 103

Gnathostoma spinigerum Owen, 1836, a contribution to the knowledge of the second intermediate hosts of 137

Gordon, R. M., and Davey, T. H. The size of aperture necessary in screen-cloth intended for the protection of dwellings in West Africa..... 413

Guinea-pig, a bartonella of..... 215

Guinea-worm larvae, some observations on 193

Hainan Island, a collection of anopheline mosquitoes from 387

„ a new tree-hole breeding *Anopheles* from 279

Hawking, F. Analysis of the trypanocidal action of trivalent arsenicals and acriflavine 313

Hawking, F. A note on the trypanocidal action of atebrin in relation to its absorption by trypanosomes 383

Hawking, F. A quantitative study of the photosensitivity induced in trypanosomes by acriflavine..... 367

Ho, C. A new tree-hole breeding *Anopheles* from the island of Hainan... 279

Ho, C. On a collection of anopheline mosquitoes from the island of Hainan 387

Ho, C. On some species of *Sarcophaga* from Java and its neighbouring islands 115

Ho, C. The significance of the female terminalia of house-flies as a grouping character 287

Ho, C., and Patton, W. S. The study of the male and female terminalia of the genus *Sarcophaga*, with illustrations of the terminalia of the *haemorrhoidalis* group..... 141

PAGE

House-flies, key to genera by female terminalia 299
" key to genera by male terminalia 309
" the significance of the female terminalia of, as a grouping character 287
Java, some species of *Sarcophaga* from... 115
Kau, L. S., and Wu, K. A note on the pathology of schistosomiasis due to *S. japonicum* among cattle in China... 129
Kau, L. S., and Wu, K. Pathological findings among pigs experimentally infected with *Fasciolopsis buskii*..... 133
King, H., Lourie, E. M., and Yorke, W. Studies in chemotherapy. XIX: Further report on new trypanocidal substances..... 177
Kingfisher, new species of nematode (*Subulura leachii*) from..... 159
Kirk, R. A laboratory study of Abyssinian louse-borne relapsing fever..... 339
Kirk, R. The non-transmission of Abyssinian louse-borne relapsing fever by the tick *Ornithodorus savignyi* and certain other blood-sucking arthropods 357
Kirshner, A. A new species of nematode (*Subulura leachii*) from a kingfisher ... 159
Kirshner, A., and Southwell, T. On the transmission of leishmaniasis..... 95
Kirshner, A., and Southwell, T. Some observations on guinea-worm larvae 193
Kondi, A., and Foy, H. A note on intracorpuscular methaemoglobin in plasmochin toxicity 249
League of Nations: fourth general report of the Malaria Commission..... 1
Leishmaniasis, transmission of 95
Lourie, E. M., Glyn-Hughes, F., and Yorke, W. Studies in chemotherapy. XVII: The action of undecane diamidine in malaria..... 103
Lourie, E. M., King, H., and Yorke, W. Studies in chemotherapy. XIX: Further report on new trypanocidal substances..... 177
Lourie, E. M., and Yorke, W. Studies in chemotherapy. XX: The preparation of strains of trypanosomes resistant to synthalin and undecane diamidine, and an analysis of their characters 201
Louse-borne relapsing fever, a laboratory study of 339

PAGE

Louse-borne relapsing fever, the non-transmission of Abyssinian louse-borne relapsing fever by the tick *Ornithodorus savignyi* and certain other blood-sucking arthropods 357
Malaria, the action of undecane diamidine in 103
" observations on the course of *Plasmodium knowlesi* infection in monkeys (*Macacus rhesus*), with notes on its treatment by (1) atebrin and (2) 1 : 11 normal undecane diamidine, together with a note on the action of the latter on bird malaria 257
" parasites, inhibitive effect of drugs upon oxygen uptake by malaria parasites (*Plasmodium knowlesi*) 77
" parasites, observations on the respiratory metabolism of ... 43
" treatment of 1
Mary Kingsley medallists i
Mary Kingsley medals, presentation of, November 23rd, 1938..... ii
Methaemoglobin, intracorpuscular, in plasmochin toxicity..... 249
Mexico, nematodes of the reptiles of..... 225
Monkeys (*Cercopithecus* sp.) infected with a strain of *Trypanosoma rhodesiense*, cerebrospinal fluid of..... 197
" (*Macacus rhesus*), *Plasmodium knowlesi* infection in, with notes on its treatment by (1) atebrin and (2) 1 : 11 normal undecane diamidine 257
Mosquitoes, fungal infections of, especially of *Anopheles costalis* 231
Musca Linnaeus 299
Myiasis, a note upon myiasis due to the larvae of *Cordylobia rodhaini* Gedoelst 431
Nematode, a new species of nematode (*Subulura leachii*) from a kingfisher ... 159
Nematodes of the reptiles of Mexico ... 225
Onchocerca volvulus Leuckart, the mouthparts of the female in *Simulium damnosum* Theobald, with special reference to the transmission of 9

PAGE

Ornithodorus savignyi, the non-transmission of Abyssinian louse-borne relapsing fever by 357

Oxygen uptake, inhibitive effect of drugs upon oxygen uptake by trypanosomes (*Trypanosoma rhodesiense*) and malaria parasites (*Plasmodium knowlesi*)..... 77

Ozolaimus ctenosauri sp. nov..... 225

„ *megatyphlon* Rudolphi, 1819... 225

Patton, W. S., and Ho, C. The study of the male and female terminalia of the genus *Sarcophaga*, with illustrations of the terminalia of the *haemorrhoidalis* group..... 141

Pattonia subgen. nov. 302

Pawan, J. L. An unusual strain of rabies virus in a vampire bat..... 35

Photosensitivity induced in trypanosomes by acriflavine, a quantitative study of 367

Pigs, pathological findings among pigs experimentally infected with *Fasciolopsis buskii* 133

Pinkerton, H., and Weinman, D. A bartonella of the guinea-pig, *Bartonella tyzzeri* sp. nov. 215

Plasmochin toxicity, intracorpuscular methaemoglobin in..... 249

„ *see also* Plasmoquine.

Plasmodium knowlesi infection in monkeys (*Macacus rhesus*), observations on the course of, with notes on its treatment by (1) atebrin and (2) 1 : 11 normal undecane diamidine 257

„ *knowlesi*, inhibitive effect of drugs upon oxygen uptake by 77

„ *knowlesi*, observations on the respiratory metabolism of 59

Plasmoquine in treatment of malaria : study of the therapeutics and prophylaxis of malaria by synthetic drugs as compared with quinine ... 1

„ *see also* Plasmochin.

Quinine in treatment of malaria : study of the therapeutics and prophylaxis of malaria by synthetic drugs as compared with quinine 1

PAGE

Rabies virus, an unusual strain of, in a vampire bat 35

Relapsing fever, a laboratory study of louse-borne relapsing fever 339

„ fever, the non-transmission of Abyssinian louse-borne relapsing fever by the tick *Ornithodorus savignyi* and certain other blood-sucking arthropods 357

Reptiles of Mexico, nematodes of..... 225

Respiratory metabolism of malaria parasites and trypanosomes, observations on 43

Roofing in the tropics..... 39

Sarcophaga, some species from Java and its neighbouring islands... 115

„ study of the male and female terminalia of the genus... 141

„ *albiceps* Meigen 119

„ *antilope* Böttcher..... 116

„ *arno* Curran 153

„ *arnoides* sp. nov..... 154

„ *auricauda* sp. nov. 123

„ *fuscicauda* Böttcher..... 119

„ *haemorrhoidalis* Meigen..... 151

„ *hirtipes* var. *orchidea* Böttcher 118

„ *knabi* Parker 118

„ *orientalis* Parker 120

„ *senior-whitei* nom. nov..... 117

„ *serrata* sp. nov. 120

„ *walshi* sp. nov..... 125

Schistosoma japonicum, a note on the pathology of schistosomiasis due to *S. japonicum* among cattle in China ... 129

Schistosomiasis due to *S. japonicum* among cattle in China, a note on the pathology of..... 129

Screencloth, the size of aperture necessary in screencloth intended for the protection of dwellings in West Africa 413

Simuliidae, Ethiopian, notes on..... 21

Simulium adersi Pomeroy 24

„ *africanum* Gibbins 24

„ *alcocki* Pomeroy 25

„ *arnoldi* Gibbins 25

„ *barnardi* sp. nov..... 22

„ *bisnovem* sp. nov..... 23

„ *blacklocki* Edwards..... 25

PAGE

Simulium bovis De Meillon..... 25
„ *cavum* nom. nov..... 26
„ *cervicornutum* Pomeroy 26
„ *damnosum* Theobald 26
„ *damnosum* Theobald, the mouth-parts of the female, with special reference to the transmission of *Onchocerca volvulus* Leuckart..... 9
„ *dentulosum* Roubaud 27
„ *elgonensis* Gibbins 27
„ *griseicolle* Becker..... 27
„ *hirsutum* Pomeroy 28
„ *impukane* De Meillon 28
„ *kauntzeum* sp. nov..... 23
„ *lepidum* De Meillon..... 28
„ *medusaeformis* Pomeroy 28
„ *neavei* Roubaud 29
„ *neireti* Roubaud 29
„ *nigritarsis* Coquillett 30
„ *palmeri* Pomeroy..... 31
„ *ruficorne* Macquart 31
„ *simplex* Gibbins 32
„ *speculiventre* Enderlein 32
„ *taylori* sp. nov..... 24
„ *tentaculum* Gibbins..... 32
„ *touffeum* Gibbins..... 32
„ *turneri* sp. nov..... 22
„ *unicornutum* Pomeroy..... 32
„ *wellmanni* Roubaud..... 33
„ *woodi* De Meillon 33

Southwell, T., and Kirshner, A. On the transmission of leishmaniasis..... 95

Southwell, T., and Kirshner, A. Some observations on guinea-worm larvae... 193

Strongyluris similis sp. nov. 227

Subulura leachii sp. nov. 159

Synthalin, preparation of strains of trypanosomes resistant to synthalin and undecane diamidine, and an analysis of their characters 201

Tansurat, P., and Daengsvang, S. A contribution to the knowledge of the second intermediate hosts of *Gnathostoma spinigerum* Owen, 1836..... 137

Terminalia, female, of house-flies as a grouping character, the significance of 287

PAGE

Transmission of leishmaniasis 95

Treatment of malaria: study of the therapeutics and prophylaxis of malaria by synthetic drugs as compared with quinine: fourth general report of the Malaria Commission of the League of Nations..... 1

Trivalent arsenicals and acriflavine, analysis of the trypanocidal action of 313

Trypanocidal action of atebrin in relation to its absorption by trypanosomes 383
„ action of trivalent arsenicals and acriflavine, analysis of..... 313
„ substances, new, report on 177

Trypanosoma congolense Broden, 1904, observations on the behaviour in laboratory animals of 425
„ *gambiense*, a third note on a strain of *T. gambiense* transmitted by *Glossina morsitans* 245
„ *rhodesiense*, cerebrospinal fluid of monkeys (*Cercopithecus* sp.) infected with a strain of 197
„ *rhodesiense*, inhibitive effect of drugs upon oxygen uptake by 77
„ *rhodesiense*, observations on the respiratory metabolism of 45
„ *rhodesiense*, a record of some complications which occurred in the course of experimental infections of African volunteers with 437

Trypanosomes, inhibitive effect of drugs upon oxygen uptake by trypanosomes (*Trypanosoma rhodesiense*) 77
„ a note on the trypanocidal action of atebrin in relation to its absorption by trypanosomes 383

PAGE

Trypanosomes, observations on the respiratory metabolism of 43
" preparation of strains of, resistant to synthalin and undecane diamidine, and an analysis of their characters..... 201
" a quantitative study of the photosensitivity induced in trypanosomes by acriflavine... 367
Undecane diamidine, the action of, in malaria 103
" diamidine, changes in the blood and urine produced by administration of..... 163
" diamidine, observations on the course of *Plasmodium knowlesi* infection in monkeys, with notes on its treatment by 1 : 11 normal undecane diamidine 257
" diamidine, preparation of strains of trypanosomes resistant to synthalin and undecane diamidine, and an analysis of their characters 201
Urine, changes in, produced by the administration of undecane diamidine 163
Vampire bat, an unusual strain of rabies virus in..... 35

PAGE

Vitamin C deficiency, arcus senilis as a sign of 333
Viviparomusca Townsend 305
Walker, A. J. Fungal infections of mosquitoes, especially of *Anopheles costalis* 231
Weinman, D., and Pinkerton, H. A bartonella of the guinea-pig, *Bartonella tyzzeri* sp. nov..... 215
West African dwellings, the size of aperture necessary in screencloth intended for the protection of..... 413
Wu, K., and Kau, L. S. A note on the pathology of schistosomiasis due to *S. japonicum* among cattle in China ... 129
Wu, K., and Kau, L. S. Pathological findings among pigs experimentally infected with *Fasciolopsis buskii*..... 133
Yorke, W., Glyn-Hughes, F., and Lourie, E. M. Studies in chemotherapy. XVII : The action of undecane diamidine in malaria 103
Yorke, W., King, H., and Lourie, E. M. Studies in chemotherapy. XIX : Further report on new trypanocidal substances..... 177
Yorke, W., and Lourie, E. M. Studies in chemotherapy. XX : The preparation of strains of trypanosomes resistant to synthalin and undecane diamidine, and an analysis of their characters..... 201

INDEX TO SPECIES NEW TO SCIENCE

PAGE

Anopheles sintonoides sp. nov..... 279
Bartonella tyzzeri sp. nov..... 215
Coelomomyces africanus sp. nov..... 239
Ozolaimus ctenosauri sp. nov..... 225
Sarcophaga arnoides sp. nov. 154
" *auricauda* sp. nov. 123
" *serrata* sp. nov..... 120
" *walshi* sp. nov..... 125

PAGE

Simulium barnardi sp. nov..... 22
" *bisnovem* sp. nov..... 23
" *kauntzeum* sp. nov..... 23
" *taylori* sp. nov..... 24
" *turneri* sp. nov..... 22
Strongyluris similis sp. nov..... 227
Subulura leachii sp. nov..... 159